Supporting Information

Global screening and genetic engineering of *Tistrella* enable sustainable production of didemnin drugs

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General methods

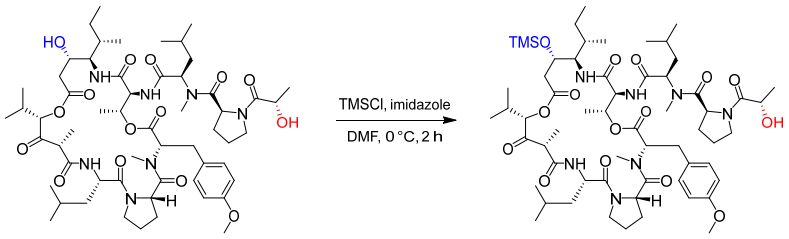
NMR spectra were recorded on a Bruker BioSpin GmbH 400 MHz spectrometer equipped with an avance Neo 400 iprobe or Bruker BioSpin GmbH 600 MHz spectrometer equipped with cryoprobe prodigy. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane. All spectra were calibrated against the (residual proton) peak of the deuterated solvent used ($CDCl_3$ at 7.26 ppm for ^{1}H and 77.06 ppm for ^{13}C). Data are reported as follows: chemical shift {multiplicity [singlet (s), doublet (d), triplet (t), quartet (q), and multiplet (m)], coupling constants [Hz], integration}. All spectra were registered at 25 °C. Reagents and chemicals were purchased from the Bide Pharmatech Ltd unless otherwise noted and used without further purification.

Semi-preparative HPLC was performed on an Agilent 1260 Infinity II equipped with a multi-wavelength UV-Vis detector (detection at 210 nm and 230 nm) using a Kinetex XB-C18 column (10 × 250 mm, 5 μm, 100Å) from Phenomenex. Mobile phase condition was set as follows: (water + acetonitrile (ACN)): 0-10 min gradient 40% to 90% ACN, 10-16 min isocratic 10% water 90% ACN, 16.10-20 min isocratic 60% water 40% ACN. Flow rate = 2.5 mL/min.

Optical rotation data were obtained on a JASCOP-2000 Polarimeter at 20 °C.

Methods for chemical synthesis

(*S*)-*N*-((*R*)-1-(((3*S*,6*R*,7*S*,10*R*,11*S*,15*R*,17*S*,20*S*,25a*S*)-10-((*S*)-*sec*-butyl)-20-isobutyl-15-isopropyl-3-(4-methoxybenzyl)-2,6,17-trimethyl-1,4,8,13,16,18,21-heptaoxo-11-((trimethylsilyl)oxy)docosahydro-15*H*-pyrrolo[2,1-*f*][1,15]dioxa[4,7,10,20]tetraazacyclotricosin-7-yl)amino)-4-methyl-1-oxopentan-2-yl)-1-((*S*)-2-hydroxypropanoyl)-*N*-methylpyrrolidine-2-carboxamide (3)



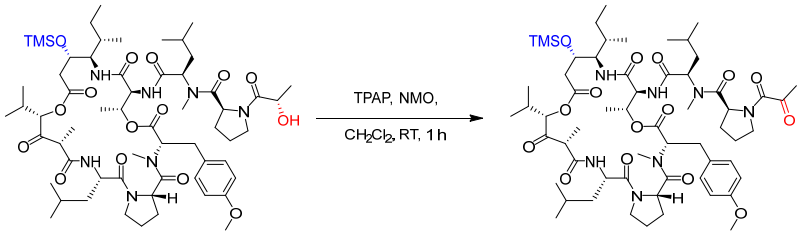
Didemnin B **2** **3**

Didemnin B (**2**) (10.0 mg, 0.009 mmol) was dissolved in anhydrous DMF (0.2 mL) and then cooled to 0 °C in an ice bath. TMSCl (1.5 mg, 0.013 mmol) and imidazole (1.2 mg, 0.018 mmol) were added. The reaction mixture was stirred at 0 °C for 2 h. After complete reaction detected by LC-MS, the reaction was quenched with water (2.0 mL) and extracted with ethyl acetate (3 × 2.0 mL). The organic layers were combined and washed with water, saturated NaCl, dried over anhydrous Na_2SO_4, filtered and evaporated in *vacuo*. The residue was used directly in next step without further purification because of the lability of TMS ether.

HRMS (ESI): calcd for $C_{60}H_{98}N_7O_{15}Si$ $[M+H]^+$ 1184.6885, found 1184.6908.

HRMS^2 (CID): calcd for $C_{45}H_{74}N_5O_{11}Si$ $[M+H]^+$ 888.5149, found 888.5173; calcd for $C_{15}H_{25}N_2O_4$ $[M+H]^+$ 297.1809, found 297.1804.

(*S*)-*N*-((*R*)-1-(((3*S*,6*R*,7*S*,10*R*,11*S*,15*R*,17*S*,20*S*,25a*S*)-10-((*S*)-*sec*-butyl)-20-isobutyl-15-isopropyl-3-(4-methoxybenzyl)-2,6,17-trimethyl-1,4,8,13,16,18,21-heptaoxo-11-((trimethylsilyl)oxy)docosahydro-15*H*-pyrrolo[2,1-*f*][1,15]dioxa[4,7,10,20]tetraazacyclotricosin-7-yl)amino)-4-methyl-1-oxopentan-2-yl)-*N*-methyl-1-(2-oxopropanoyl)pyrrolidine-2-carboxamide (4)



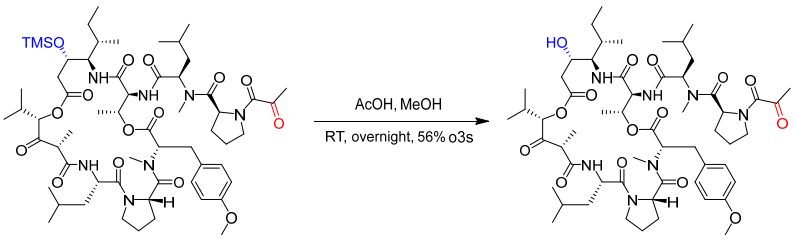
3 4

TPAP (1.0 mg, 0.003 mmol) is added in one portion to a stirred mixture of **3** (10.6 mg, 0.009 mmol), NMO (1.6 mg, 0.013 mmol) in anhydrous CH_2Cl_2 (0.2 mL) at room temperature under inert atmosphere. After complete reaction detected by LC-MS, the mixture was diluted with CH_2Cl_2 (0.8 mL) and then washed with 10% aqueous $NaHSO_3$ (1.0 mL), saturated NaCl (1.0 mL), and dried over anhydrous Na_2SO_4. The organic solvent was filtered, evaporated in *vacuo* and use directly in next step without further purification.

HRMS (ESI): calcd for $C_{60}H_{95}N_7O_{15}SiNa$ $[M+Na]^+$ 1204.6548, found 1204.6552.

HRMS^2 (CID): calcd for $C_{45}H_{74}N_5O_{11}Si$ $[M+H]^+$ 888.5149, found 888.5149; calcd for $C_{15}H_{23}N_2O_4$ $[M+H]^+$ 295.1652, found 295.1649.

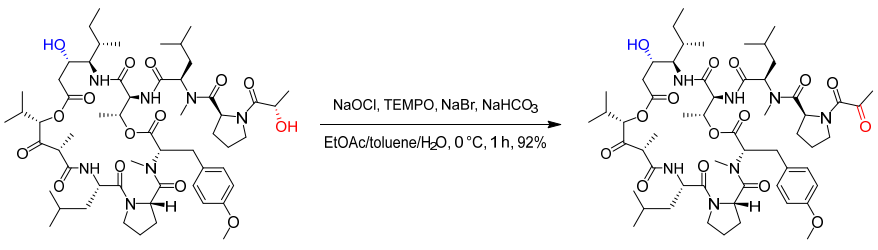
(*S*)-*N*-((*R*)-1-(((3*S*,6*R*,7*S*,10*R*,11*S*,15*R*,17*S*,20*S*,25a*S*)-10-((*S*)-*sec*-butyl)-11-hydroxy-20-isobutyl-15-isopropyl-3-(4-methoxybenzyl)-2,6,17-trimethyl-1,4,8,13,16,18,21-heptaoxodocosahydro-15*H*-pyrrolo[2,1-*f*][1,15]dioxa[4,7,10,20]tetraazacyclotricosin-7-yl)amino)-4-methyl-1-oxopentan-2-yl)-*N*-methyl-1-(2-oxopropanoyl)pyrrolidine-2-carboxamide (1, Plitidepsin)



4 Plitidepsin **1**

A solution of **4** (8.0 mg, 0.007 mmol) in 0.2 mL of MeOH was treated with AcOH (4.1 mg, 0.068 mmol) and stirred overnight. The mixture was then diluted with EtOAc (1.0 mL), washed with H_2O (1.0 mL), saturated $NaHCO_3$ (1.0 mL), and saturated NaCl (1.0 mL). The organic layer was dried over Na_2SO_4 and removed under reduced pressure to give oil residue. The crude product was purified by preparative HPLC to give **2** as white solid (5.6 mg, 56%).

Chemical synthesis of plitidepsin (1) by regioselective oxidation



Didemnin B **2** Plitidepsin **1**

To a stirred solution of didemnin B (20.0 mg, 0.018 mmol) in a mixture of toluene/EtOAc (1:1, 0.2 mL) at 0 °C, a solution of NaBr (1.9 mg, 0.019 mmol) in water (20 µl) was

added followed by TEMPO (0.3 mg, 0.002 mmol). To the resulting biphasic system, a solution of NaOCl (1.5 mg, 0.02 mmol) and $NaHCO_3$ (4.5 mg, 0.054 mmol) in H_2O (60 µL) was added dropwise under vigorous stirring at 0 °C over a period of 1 h. After stirring for further 15 min at 0 °C, EtOAc (0.9 mL) and H_2O (0.3 mL) were added. The aqueous layer was separated and extracted with EtOAc (0.5 mL). The combined organic layers were washed with 10% aqueous $NaHSO_3$ (1.0 mL) containing KI (1.0 mg), 10% aqueous $Na_2S_2O_3$ (1.0 mL), saturated NaCl (1.0 mL) and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure and the crude product was purified by preparative HPLC (Kinetex 5 µm XB-C18 100 Å, 250 × 10.0 mm) to give **1** as white solid (18.4 mg, 92%).

$[\alpha]_D^{20}$ = -45.6 (*c* 0.57, CH_2Cl_2).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.85 (d, 1H, *J* = 9.2 Hz), 7.80 (d, 1H, *J* = 9.2 Hz), 7.60 (d, 1 H, *J* = 5.8 Hz), 7.19 (t, 1H, *J* = 9.4 Hz), 7.08 (d, 2H, *J* = 8.6 Hz), 7.07 (d, 2H, *J* = 8.6 Hz), 7.05 (d, 1H, *J* = 6.6 Hz), 6.85 (d, 2H, *J* = 8.6 Hz), 6.84 (d, 2H, *J* = 8.6 Hz), 5.39 (dd, 1H, *J* = 3.8, 11.3 Hz), 5.33-5.27 (m, 2H), 5.21-5.15 (m, 3H), 5.12-5.08 (m, 1H), 4.83-4.77 (m, 2H), 4.71 (t, 1H, *J* = 7.0 Hz), 4.66 (dd, 1H, *J* = 2.2, 6.5 Hz), 4.64-4.60 (m, 2H), 4.57 (dd, 1H, *J* = 2.0, 5.6 Hz), 4.22 (q, 1H, *J* = 6.8, 13.7 Hz), 4.18 (q, 1H, *J* = 6.8, 13.7 Hz), 4.13-4.02 (m, 4H), 4.02-3.97 (m, 1H), 3.89-3.83 (m, 1H), 3.82-3.76 (m, 7H), 3.74-3.67 (m, 3H), 3.62-3.56 (m, 4H), 3.35 (dd, 2H, *J* = 4.1, 14.3 Hz), 3.24 (dd, 2H, *J* = 7.2, 16.7 Hz), 3.20-3.13 (m, 5H), 3.12-3.09 (m, 3H), 2.69-2.55 (m, 8H), 2.53 (s, 3H), 2.52 (s, 3H), 2.41-2.32 (m, 3H), 2.19-2.09 (m, 5H), 2.06-1.88 (m, 8H), 1.83-1.74 (m, 6H), 1.72-1.56 (m, 18H), 1.45-1.39 (m, 8H), 1.34-1.31 (m, 6H), 1.26-1.18 (m, 8H), 0.95-

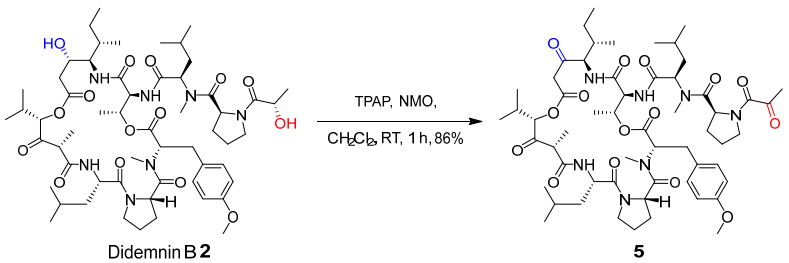
0.84 (m, 24H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 205.1, 204.9, 201.4, 197.3, 173.2, 172.5, 172.0, 171.8, 171.3, 170.8, 169.8, 169.7, 169.5, 168.5, 161.5, 158.8, 130.5, 130.0, 129.9, 114.3, 81.7, 81.6, 70.8, 70.5, 68.1, 68.0, 59.1, 57.9, 57.6, 57.4, 57.3, 55.8, 55.4, 54.8, 49.7, 49.0, 48.6, 47.2, 41.5, 41.4, 38.9, 38.8, 36.6, 36.4, 34.2, 34.1, 31.5, 30.9, 28.1, 27.4, 27.3, 27.2, 26.4, 25.2, 25.0, 24.9, 24.8, 24.1, 24.0, 23.7, 23.6, 22.5, 21.5, 21.4, 21.1, 21.0, 18.8, 18.7, 17.1, 17.0, 16.4, 16.1, 15.4, 14.9, 14.8, 11.8.

HRMS (ESI): calcd for $C_{57}H_{87}N_7O_{15}Na$ $[M+Na]^+$ 1132.6152, found 1132.6153.

HRMS^2 (CID): calcd for $C_{42}H_{66}N_5O_{11}$ $[M+H]^+$ 816.4753, found 816.4761; calcd for $C_{15}H_{23}N_2O_4$ $[M+H]^+$ 295.1652, found 295.1651.

(*S*)-*N*-((*R*)-1-(((3*S*,6*R*,7*S*,10*R*,15*R*,17*S*,20*S*,25a*S*)-10-((*S*)-*sec*-butyl)-20-isobutyl-15-isopropyl-3-(4-methoxybenzyl)-2,6,17-trimethyl-1,4,8,11,13,16,18,21-octaoxodocosahydro-15*H*-pyrrolo[2,1-*f*][1,15]dioxa[4,7,10,20]tetraazacyclotricosin-7-yl)amino)-4-methyl-1-oxopentan-2-yl)-*N*-methyl-1-(2-oxopropanoyl)pyrrolidine-2-carboxamide (**5**)



Didemnin B **2** **5**

TPAP (1.0 mg, 0.003 mmol) is added in one portion to a stirred mixture of didemnin B (18.0 mg, 0.016 mmol), NMO (5.7 mg, 0.049 mmol) in anhydrous CH_2Cl_2 (0.2 mL) at

room temperature under inert atmosphere. After complete reaction detected by LC-MS, the mixture was diluted with CH_2Cl_2 (0.8 mL) and then washed with 10% aqueous $NaHSO_3$ (1.0 mL), H_2O (1.0 mL), saturated NaCl (1.0 mL), and dried over anhydrous Na_2SO_4. The organic solvent was filtered, evaporated in *vacuo* and purified by preparative HPLC to give **5** as white solid (15.4 mg, 86%).

$[\alpha]_D^{20}$ = -50.9 (*c* 0.33, CH_2Cl_2).

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.00 (d, 2H, *J* = 9.3 Hz), 7.96-7.90 (m, 1H), 7.85 (d, 1H, *J* = 10.3 Hz), 7.58 (d, 1 H, *J* = 6.0 Hz), 7.09-7.04 (m, 4H), 6.87-6.82 (m, 4H), 5.40 (dd, 1H, *J* = 3.8, 11.3 Hz), 5.34-5.28 (m, 2H), 5.22-5.18 (m, 2H), 5.12-5.08 (m, 1H), 4.93 (dd, 1H, *J* = 2.3, 6.9 Hz), 4.86-4.82 (m, 1H), 4.78 (t, 2H, *J* = 11.0 Hz), 4.72 (t, 1H, *J* = 7.2 Hz), 4.61-4.51 (m, 3H), 4.30 (t, 1H, *J* = 6.6 Hz), 4.19 (d, 1H, *J* = 3.0 Hz), 4.16 (d, 1H, *J* = 3.0 Hz), 4.09 (q, 1H, *J* = 6.7, 13.6 Hz), 4.04 (q, 1H, *J* = 6.6, 13.6 Hz), 4.02-3.97 (m, 2H), 3.89-3.83 (m, 2H), 3.82-3.76 (m, 6H), 3.74-3.67 (m, 3H), 3.62-3.56 (m, 4H), 3.34 (dd, 2H, *J* = 4.4, 14.5 Hz), 3.27-3.18 (m, 2H), 3.12 (s, 3H), 3.07 (s, 3H), 2.64-2.60 (m, 5H), 2.54-2.50 (m, 5H), 2.42-2.28 (m, 3H), 2.27-2.12 (m, 6H), 2.09-1.86 (m, 10H), 1.83-1.56 (m, 24H), 1.40 (t, 7H, *J* = 6.6 Hz), 1.32 (d, 6H, *J* = 7.0 Hz), 1.26-1.18 (m, 8H), 0.95-0.84 (m, 42H).

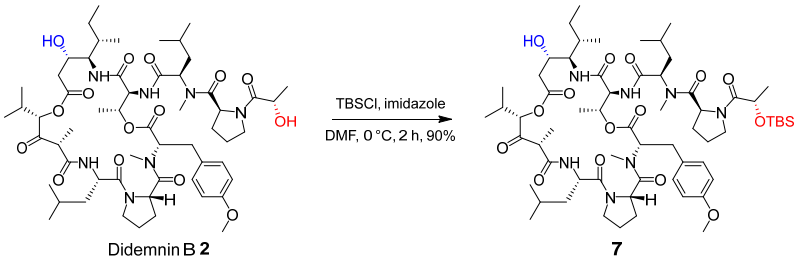
^{13}C NMR (151 MHz, $CDCl_3$) δ 205.3, 204.9, 202.9, 201.3, 197.3, 173.3, 172.5, 172.1, 171.6, 171.5, 171.0, 170.2, 169.9, 168.3, 166.7, 166.6, 164.9, 161.6, 161.1, 160.4, 158.8, 131.0, 130.5, 129.8, 129.0, 114.3, 81.8, 77.4, 77.2, 77.0, 70.8, 70.6, 66.8, 66.7, 65.7, 62.8, 62.5, 59.1, 57.7, 57.4, 56.8, 55.4, 54.9, 54.8, 50.3, 50.3, 49.6, 49.0, 48.6,

47.3, 45.0, 44.8, 41.2, 38.9, 38.8, 36.6, 36.3, 34.0, 31.7, 31.6, 31.5, 31.4, 30.7, 29.8, 28.3, 28.1, 27.4, 27.2, 26.4, 26.2, 26.1, 25.5, 25.1, 24.9, 24.8, 24.0, 23.7, 23.6, 22.5, 21.5, 21.4, 21.1, 21.0, 18.8, 16.9, 16.3, 16.0, 15.8, 15.0, 14.9, 11.3, 11.2.

HRMS (ESI): calcd for $C_{57}H_{85}N_7O_{15}Na$ $[M+Na]^+$ 1130.5996, found 1130.6003.

HRMS^2 (CID): calcd for $C_{42}H_{64}N_5O_{11}$ $[M+H]^+$ 814.4597, found 814.4606; calcd for $C_{15}H_{23}N_2O_4$ $[M+H]^+$ 295.1652, found 295.1651.

(*S*)-*N*-((*R*)-1-(((3*S*,6*R*,7*S*,10*R*,11*S*,15*R*,17*S*,20*S*,25a*S*)-10-((*S*)-*sec*-butyl)-11-hydroxy-20-isobutyl-15-isopropyl-3-(4-methoxybenzyl)-2,6,17-trimethyl-1,4,8,13,16,18,21-heptaoxodocosahydro-15*H*-pyrrolo[2,1-*f*][1,15]dioxa[4,7,10,20]tetraazacyclotricosin-7-yl)amino)-4-methyl-1-oxopentan-2-yl)-1-((*S*)-2-((*tert*-butyldimethylsilyl)oxy)propanoyl)-*N*-methylpyrrolidine-2-carboxamide (**7**)



Didemnin B **2** **7**

Didemnin B (20.0 mg, 0.018 mmol) was dissolved in anhydrous DMF (0.2 mL) and then cooled to 0 °C in an ice bath. TBSCl (5.4 mg, 0.036 mmol) and imidazole (3.7 mg, 0.054 mmol) were added. The reaction mixture was stirred at 0 °C for 2 h. After complete reaction detected by LC-MS, the reaction was quenched with water (2.0 mL) and extracted with ethyl acetate (3 × 2.0 mL). The organic layers were combined and

washed with water, saturated NaCl, dried over anhydrous Na_2SO_4, filtered and evaporated in *vacuo*. The residue was purified by semi-preparative HPLC to give compound **7** as a white solid (19.9 mg, 90%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, 1H, *J* = 9.2 Hz), 7.83 (d, 1H, *J* = 5.8 Hz), 7.19 (d, 1H, *J* = 9.7 Hz), 7.07 (d, 2H, *J* = 8.0 Hz), 6.84 (d, 2H, *J* = 8.0 Hz), 5.38 (dd, 1H, *J* = 3.5, 11.2 Hz), 5.25-5.15 (m, 2H), 4.80 (t, 1H, *J* = 10.0 Hz), 4.67 (t, 1H, *J* = 7.3 Hz), 4.61 (t, 1H, *J* = 6.0 Hz), 4.56 (d, 1H, *J* = 5.3 Hz), 4.49 (q, 1H, *J* = 6.5, 13.3 Hz), 4.23 (q, 1H, *J* = 6.8, 13.8 Hz), 4.15-4.01 (m, 2H), 3.93-3.86 (m, 1H), 3.79 (s, 3H), 3.76-3.66 (m, 2H), 3.63-3.52 (m, 2H), 3.36 (dd, 1H, *J* = 3.8, 14.2 Hz), 3.25-3.17 (m, 2H), 3.14 (s, 3H), 2.64 (dd, 1H, *J* = 10.8, 16.9 Hz), 2.55 (s, 3H), 2.39-2.31 (m, 1H), 2.23-1.99 (m, 6H), 1.97-1.86 (m, 3H), 1.85-1.65 (m, 9H), 1.41 (t, 8H, *J* = 7.0 Hz), 1.33 (d, 3H, *J* = 6.7 Hz), 1.27-1.17 (m, 5H), 0.96-0.84 (m, 30H), 0.10 (s, 6H).

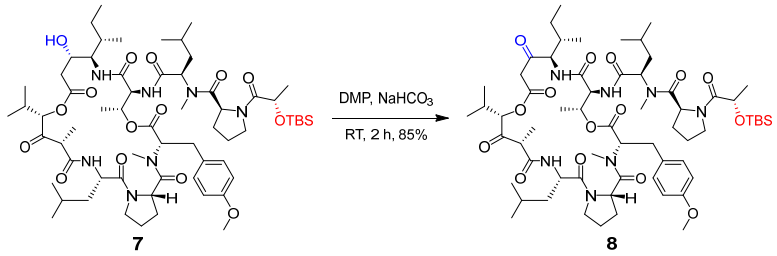
^{13}C NMR (101 MHz, $CDCl_3$) δ 205.2, 173.6, 172.6, 172.4, 172.2, 171.3, 170.7, 170.0, 169.8, 168.2, 158.7, 130.5, 130.2, 114.2, 81.7, 71.1, 70.0, 68.2, 66.7, 58.1, 57.3, 56.9, 55.8, 55.4, 54.7, 49.8, 49.6, 47.6, 47.1, 41.4, 38.9, 38.9, 36.6, 34.3, 34.1, 31.6, 31.5, 28.2, 28.1, 27.1, 26.3, 26.0, 25.2, 25.0, 24.9, 24.0, 23.6, 21.6, 21.1, 20.6, 18.7, 18.3, 17.1, 16.6, 15.4, 15.0, 11.7, -4.3.

HRMS (ESI): calcd for $C_{63}H_{103}N_7O_{15}SiNa$ $[M+Na]^+$ 1248.7174, found 1248.7173.

HRMS^2 (CID): calcd for $C_{42}H_{66}N_5O_{11}$ $[M+H]^+$ 816.4753, found 816.4752; calcd for $C_{21}H_{39}N_2O_4Si$ $[M+H]^+$ 411.2674, found 411.2662.

(*S*)-*N*-((*R*)-1-(((3*S*,6*R*,7*S*,10*R*,15*R*,17*S*,20*S*,25a*S*)-10-((*S*)-*sec*-butyl)-20-isobutyl-

15-isopropyl-3-(4-methoxybenzyl)-2,6,17-trimethyl-1,4,8,11,13,16,18,21-octaoxodocosahydro-15*H*-pyrrolo[2,1-*f*][1,15]dioxa[4,7,10,20]tetraazacyclotricosin-7-yl)amino)-4-methyl-1-oxopentan-2-yl)-1-((*S*)-2-((*tert*-butyldimethylsilyl)oxy)propanoyl)-*N*-methylpyrrolidine-2-carboxamide (**8**)



7 **8**

To a solution of **7** (15.0 mg, 0.012 mmol) and $NaHCO_3$ (1.5 mg, 0.018 mmol) in anhydrous CH_2Cl_2 (0.2 mL) was added Dess-Martin periodinane (7.8 mg, 0.018 mmol). The mixture was stirred at room temperature for 2 h. After complete reaction detected by LC-MS, the reaction was further diluted with CH_2Cl_2 (1.0 mL) and washed with H_2O (1.0 mL), saturated NaCl (1.0 mL). The organic solvent was dried over anhydrous Na_2SO_4, filtered and removed under reduced pressure. The residue was re-dissolved in acetonitrile (1.0 mL) and purified by semi-preparative HPLC to give the title compound **8** as white solid (12.7 mg, 85%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, 1H, *J* = 9.2 Hz), 7.84-7.77 (m, 1H), 7.08 (d, 2H, *J* = 8.0 Hz), 6.85 (d, 2H, *J* = 8.0 Hz), 5.40 (dd, 1H, *J* = 3.5, 11.5 Hz), 5.31-5.22 (m, 1H), 5.20 (d, 1H, *J* = 3.1 Hz), 4.85-4.74 (m, 2H), 4.67 (t, 1H, *J* = 7.0 Hz), 4.57 (q, 1H, *J* = 6.7, 14.7 Hz), 4.50 (q, 1H, *J* = 4.9, 11.3 Hz), 4.22-4.01 (m, 3H), 3.92-3.85 (m, 1H), 3.79 (s, 3H), 3.76-3.68 (m, 2H), 3.64-3.51 (m, 2H), 3.35 (dd, 1H, *J* = 3.7, 14.1 Hz), 3.27-

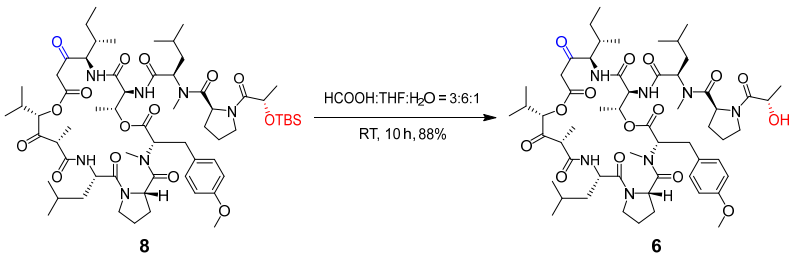
3.18 (m, 1H), 3.13 (s, 3H), 2.60 (s, 3H), 2.36-2.27 (m, 1H), 2.23-1.99 (m, 6H), 1.97-1.86 (m, 3H), 1.85-1.50 (m, 11H), 1.41 (t, 7H, *J* = 6.7 Hz), 1.32 (d, 3H, *J* = 6.7 Hz), 1.29-1.22 (m, 3H), 0.96-0.84 (m, 30H), 0.10 (s, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 205.0, 203.6, 173.6, 172.4, 172.2, 171.5, 170.9, 170.1, 168.0, 166.8, 158.8, 130.5, 130.1, 114.3, 81.7, 71.1, 70.0, 66.9, 62.4, 57.7, 57.4, 56.9, 55.4, 54.8, 50.2, 49.6, 47.6, 47.3, 45.0, 41.2, 38.9, 36.5, 36.3, 33.9, 31.7, 31.5, 28.2, 26.3, 26.2, 26.0, 25.5, 25.0, 24.9, 24.0, 23.6, 21.6, 21.1, 20.6, 18.8, 18.4, 16.9, 16.5, 15.8, 15.0, 11.3, -4.3.

HRMS (ESI): calcd for $C_{63}H_{101}N_7O_{15}SiNa$ $[M+Na]^+$ 1246.7017, found 1246.7035.

HRMS^2 (CID): calcd for $C_{42}H_{64}N_5O_{11}$ $[M+H]^+$ 814.4597, found 814.4589; calcd for $C_{21}H_{39}N_2O_4Si$ $[M+H]^+$ 411.2674, found 411.2659.

(*S*)-*N*-((*R*)-1-(((3*S*,6*R*,7*S*,10*R*,15*R*,17*S*,20*S*,25a*S*)-10-((*S*)-*sec*-butyl)-20-isobutyl-15-isopropyl-3-(4-methoxybenzyl)-2,6,17-trimethyl-1,4,8,11,13,16,18,21-octaoxodocosahydro-15*H*-pyrrolo[2,1-*f*][1,15]dioxa[4,7,10,20]tetraazacyclotricosin-7-yl)amino)-4-methyl-1-oxopentan-2-yl)-1-((*S*)-2-hydroxypropanoyl)-*N*-methylpyrrolidine-2-carboxamide (**6**)



8 **6**

A solution of compound **8** (10.0 mg, 0.008 mmol) in a mixture of HCOOH-THF-H_2O (0.4 mL, 3:6:1) was stirred at room temperature for 9 h. After complete reaction detected by LC-MS, the mixture was cooled to 0 °C and neutralized with saturated

aqueous $NaHCO_3$ (0.4 mL). After that, the mixture was extracted with ethyl acetate (3 × 1.0 mL) and the combined organic layers were washed with saturated NaCl (2.0 mL), dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The residue was re-dissolved in acetonitrile (1.0 mL) and purified by semi-preparative HPLC to give the title compound **6** as a white solid (8.0 mg, 88%).

$[\alpha]_D^{20}$ = -31.0 (*c* 0.5,CH_2Cl_2).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97-7.86 (t, 1H), 7.65 (d, 1H, *J* = 5.3 Hz), 7.07 (d, 2H, *J* = 8.0 Hz), 6.85 (d, 2H, *J* = 8.0 Hz), 5.46-5.33 (m, 2H), 5.19 (d, 1H, *J* = 3.4 Hz), 4.85-4.74 (m, 3H), 4.65-4.52 (m, 2H), 4.44-4.33 (m, 1H), 4.15-4.05 (m, 2H), 3.79 (s, 3H), 3.76-3.53 (m, 5H), 3.44-3.32 (m, 2H), 3.25-3.18 (m, 1H), 3.12 (s, 3H), 2.61 (s, 3H), 2.40-2.11 (m, 6H), 2.09-1.89 (m, 5H), 1.85-1.50 (m, 11H), 1.41 (t, 7H, *J* = 6.8 Hz), 1.32 (d, 3H, *J* = 6.8 Hz), 1.29-1.22 (m, 3H), 0.96-0.84 (m, 30H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 204.7, 203.7, 174.1, 173.0, 172.0, 171.5, 171.0, 170.2, 169.8, 168.5, 166.8, 158.8, 130.5, 130.0, 114.3, 81.7, 70.6, 66.8, 66.2, 62.4, 57.5, 57.3, 56.9, 55.4, 55.0, 50.1, 49.6, 47.3, 47.2, 44.9, 41.3, 38.8, 36.3, 36.2, 33.9, 31.4, 31.3, 29.8, 28.5, 28.2, 26.3, 26.1, 25.5, 25.0, 23.9, 23.5, 21.5, 21.1, 20.4, 18.8, 17.0, 16.4, 15.7, 14.9, 11.4.

HRMS (ESI): calcd for $C_{57}H_{87}N_7O_{15}Na$ $[M+Na]^+$ 1132.6152, found 1132.6182.

HRMS^2 (CID): calcd for $C_{42}H_{64}N_5O_{11}$ $[M+H]^+$ 814.4597, found 814.4620; calcd for $C_{15}H_{25}N_2O_4$ $[M+H]^+$ 297.1809, found 297.1811.

Supplementary Table 2 *Tistrella* strain collection from Marine Culture Collection China.

Designated names	Names	Source	Latitude	Longitude	Location
L1	*Tistrella mobilis* MCCC1A01045	marine sediment	NA	NA	Indian Ocean
L2	*Tistrella mobilis* MCCC1A01199	marine sediment	0.13	-23.62	Atlantic
L3	*Tistrella mobilis* MCCC1A01369	marine water	24.47	118.08	South China Sea
L4	*Tistrella mobilis* MCCC1A01462	marine water	6.42	95.30	Indian Ocean
L5	*Tistrella mobilis* MCCC1A02017	marine water	-31.07	58.99	Indian Ocean
L6	*Tistrella mobilis* MCCC1A02139	marine water	-26.73	66.80	Indian Ocean
L7	*Tistrella mobilis* MCCC1A02427	marine water	-7.90	-16.08	Atlantic
L8	*Tistrella mobilis* MCCC1A02807	marine water	35.18	121.67	Yellow Sea of China
L9	*Tistrella mobilis* MCCC1A02962	marine water	36.09	120.28	Yellow Sea of China
L10	*Tistrella mobilis* MCCC1A03759	marine water	20.87	117.97	South China Sea
L11	*Tistrella mobilis* MCCC1A03762	marine water	20.87	117.97	South China Sea
L12	*Tistrella bauzanensis* MCCC1A07333	marine water	53.32	169.95	Arctic Ocean
L13	*Tistrella bauzanensis* MCCC1A07475	marine water	67.50	-169.00	Arctic Ocean
L14	*Tistrella mobilis* MCCC1A11787	marine water	NA	NA	South China Sea
L15	*Tistrella mobilis* MCCC1A17629	marine water	42.65	117.70	South China Sea
L16	*Tistrella bauzanensis* MCCC1A18574	soil	46.52	11.35	Italy
L17	*Tistrella mobilis* MCCC1A11766	wastewater	13.74	100.52	Thailand

Supplementary Table 3 ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (101 MHz, $CDCl_3$) for didemnin B (**2**).

	Position	δ_C	δ_H (*J* in Hz)		Position	δ_C	δ_H (*J* in Hz)
isoSta	$C2H_a$	38.8	2.66, m	*N,O*-	CO	168.5	
	$C2H_b$		3.27, m	diMeTyr	C2H	66.5	3.58, m
	C3H	68.0	4.06, m		C3Ha	33.9	3.18, m
	C4H	55.3	4.10, m		C3Hb		3.38, m
	C5H	34.0	1.85, m		C4	130.0	
	C5-CH3	14.7	0.91, m		C5H	130.3	7.07 (d, 8.1)
	$C6H_a$	27.1	1.43, m		C6H	114.1	6.85 (d, 8.1)
	$C6H_b$		1.20, m		C7	158.6	
	C7H3	11.7	0.92, m		OCH3	55.3	3.80 (s)
	NH		7.22, d (9.6)		NCH3	38.7	2.56 (s)
Hip	CO	169.7		Thr	CO	169.4	
	C2H	49.5	4.25, q (6.7)		CαH	57.7	4.55, m
	C2-CH3	15.3	1.33, d (6.8)		CβH	70.4	5.41, m
	C3	205.0			CγH3	16.3	1.39, m
	C4H	81.5	5.19, d (3.2)		NH		7.66 (d, 5.0)
	C5H	31.3	2.36, m	D-MeLeu	CO	171.7	
	C5-CH3	16.9	0.88, m		CαH	54.9	5.38, m
	C6H3	18.6	0.91, m		$C\beta H_a$	36.2	1.70, m
Leu	CαH	49.5	4.81, m		$C\beta H_b$		1.84, m
	CβHa	41.3	1.22, m		CγH	24.9	1.43, m
	CβHb		1.61, m		CδH3	21.4	0.87, m
	CγH	24.9	1.52, m		CδH3	23.4	0.90, m
	CδH3	20.9	0.91, m		NCH3	31.3	3.15 (s)
	CδH3	23.7	0.94, m	Pro^2	CO	172.9	
	NH		7.82 (d, 9.3)		CαH	56.7	4.74, m
Pro^1	CO	170.6			CβHa	28.4	1.98, m
	CαH	57.2	4.64, m		CβHb		2.22, m
	CβHa	27.9	1.77, m		CγHa	26.0	1.97, m
	CβHb		2.14, m		CγHb		2.23, m
	CγHa	25.0	2.04, m		CδHa	47.0	3.57, m
	CγHb		2.14, m		CδHb		3.68, m
	CδHa	47.0	3.61, m	Lac	CO	173.9	
	CδHb		3.70, m		CαH	66.0	4.39, q (6.6)
					CβH3	20.2	1.39, m

Supplementary Table 4 Comparison of specific rotation of didemnin B (**2**). Entry i and ii are reported specific rotation from the literatures, and entry iii is the specific rotation for the isolated didemnin B in this study, which was measured on a JASCO P-2000 Polarimeter at 20 °C in CH_2Cl_2.

Entry		Temperature (°C)	Specific Rotation (CH_2Cl_2)
i	didemnin B	25	-78 (6.91) [3]
ii	didemnin B	25	-83 (0.3) [4]
iii	didemnin B	20	-68 (0.53)

Supplementary Table 5 Cytotoxicity of plitidepsin (1), didemnin B (2), 5 and 6 against cancer cell lines HCT116 and RKO. Docetaxel was used as positive control.

Cell Line	Name	IC_{50} (nM)	SD
HCT116	docetaxel	69.95	20.4963
	didemnin B (**2**)	13.07	0.8407
	plitidepsin (**1**)	28.36	3.3509
	5	1027.90	126.6749
	6	571.80	27.5102
RKO	docetaxel	16.51	11.80
	didemnin B (**2**)	22.53	14.05
	plitidepsin (**1**)	24.02	6.65
	5	1534.37	723.90
	6	1113.47	322.21

Supplementary Table 6 The inhibition of didemnin B (2) on the SARS-COV-2 main protease (3CL protease)**.** Ebselen was used as positive control. The Assay Kit was purchased from Beyotime Biotechnology (China) and used followed its instruction.

Entry	Blank control	100% enzymatic control	Ebselen (2 μM)	didemnin B (1 μM)	didemnin B (10 μM)	didemnin B (100 μM)
A	3291	50630	27122	47966	50502	50809
B	3167	50495	26114	50038	49872	49826
C	2926	47244	25720	49952	49952	49952
Inhibition ratio				0.00%	-0.01%	-0.01%

Supplementary Table 7 Strains and plasmids used in this study.

Strains	**Description**	**Source**
E. coli DH5α	Host for general cloning	Sangon Biotech
E. coli S17-1	Donor strain for conjugation	ATCC
E. coli ET12567/pUB307	Donor strain for conjugation	ATCC
S. coelicolor M1152	Host for the expression of BGC	
Tistrella mobilis L17	Didemnins producing strain	This study
T. mobilis L17/Δ*didB556-826*	A *did B* gene mutated strain with the base pairs from 556 to 826 are deleted	This study
T. mobilis L17::*attB*	A mutant containing the ΦC31 phage attachment site *attB* that is inserted into the gene of *locus_0167*	This study
T. mobilis L17::*attB*/pTZX01	A mutant containing a second copy of didemnin biosynthetic gene cluster	This study
Plasmids	**Description**	**Source**
pCAP01	Plasmid construction, Kanamycin resistance, *oriT*, phiC31, *attP*	[1]
pJZ001	Plasmid construction, Gentamycin resistance, *oriT*	[1]
pJZ002	Plasmid construction, temperature-sensitive *oriC*	[2]
pTHZ000	A plasmid for making gene deletion in *T. mobilis*, Gentamycin resistance, *oriT*, phiC31, *attP, sacB*	This study
pTHZ001	A plasmid for making gene deletion in *T. mobilis* with a MCS, Gentamycin resistance, *oriT*, phiC31, *attP, sacB*	This study
pTHZ001::*didB*(556-826)	pTZ002 derivative for deleting the gene *didB*(556-826) in *T. mobilis* L17	This study
pTHZ001::*0167attB*	pTZ002 derivative for introducing *attB* in *T. mobilis* L17	This study
pTZX001	pMSBBAC1 derivative containing a 97 kb insert that covers the whole didemnin BGC	This study

Supplementary Table 8 Primers used in this study.

Name	Sequence
JZ002_F	TTTTCTTTTATTGTTTGTTAGTCTTGATGCTTCACTGATAGATACAAGAGCCATAAG
JZ002_R	CCCACGACCCCTACGTACAGGCAGGTCTGTTTGCCAGGAACTGCTGAACAGCAAAAAG
JZ001_F	ACGTAGGGGTCGTGGGCCACGAAGGCGTGCACGAGTACTCCGCGGCGTTGTGACAAT
JZ001_R	AACCTGCCATAGGCCGGCCGAATTGACATAAGCCTGTTCGGTTCG
CAP01_F	TTCGGCCGGCCTATGGCAGGTTGGGCGTCGCTTGGTCGGTC
CAP01_R	TTCTTCGTCTTGCATGCCTGCAGGTCGACGGATCTTTTCCGCTG
hr2800_apali_LF	TGGGCCACGAAGGCGTGCACGATGCGACCGCCTGGGTCGCGGACGACCTG
hr2800_hindiii_LR	GGAAGGGCAAAGCTTCAGCCCGTGCAGATCCGGCGCACAGCGGAACAGGC
hr2800_hindiii_RF	CGGGCTGAAGCTTTGCCCTTCCGGATCAGCCCCGCGCGGACGGAAGCCCT
hr2800_xbai_RR	CTATACTTTCTAGAAGGATGCAGGCCCAGAGTGCCTCGATATGGCGGGAGAGAT
hr0167attB_apali_LF	ACGAAGGCGTGCACCAGTGAGGCCCTGAAAGTGGCTGGCATCGTGCGCGATGCCGGTG
hr0167attB_hindiii_LR	ATCGAAGCTTTGCGTCTGCTGCGGCGGGCCGGCCGGTGAACGATCGGC
hr0167attB_kpni_RF	ATCGGGTACCTCGCCCGGCCCGCCGCCGGCGCAACTTCGCCTGGACGCGGAC
hr0167attB_xbai_RR	CTATACTTTCTAGAAATCCGAAAAACAAATTGCATTCATTTATGTCACATCGTC
0167attB_F	AGCTTCGGTGCGGGTGCCAGGGCGTGCCCTTGGGCTCCCCGGGCGCGTACTCCACCGGTAC
0167attB_R	CGGTGGAGTACGCGCCCGGGGAGCCCAAGGGCACGCCCTGGCACCCGCACCGA
FDB-F1	AACACCCCCTCGCAGGTTGC
FDB-R2	AAATCCGGTGGGCCGAAATGCG
FDB-F3	ACCACAGCATGACCAATGC
FDB-R4	ACCGCATGGATATGGTCGCCA
FDB-F5	TGAGCTGGGACGACGACGAAGA
FDB-R6	ATCGGTCTAAAGCCCCGAATG

Supplementary Table 9 The sequence of synthesized *sacB* gene.

TGCAGGCATGCAAGACGAAGAATCCATGGGTATGGACAGATCCTCTTTAGgcccgtagtctgcaaatcctttatgattttctatcaaacaaaagaggaaaatagaccagttgcaatccaa
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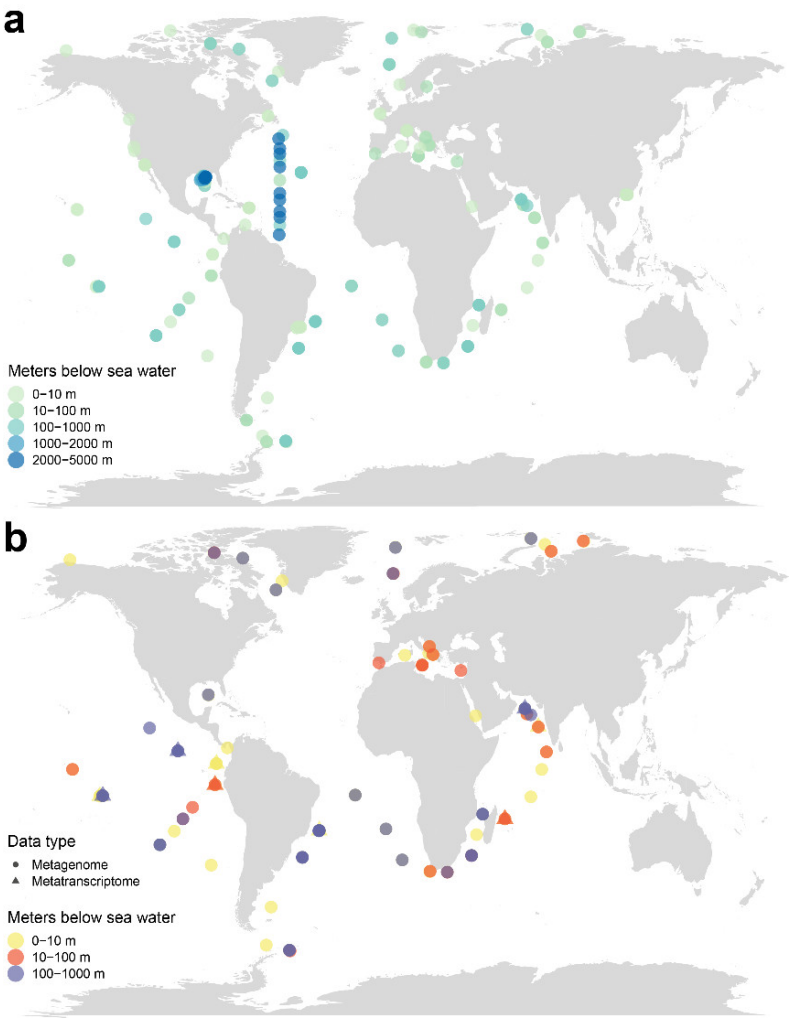
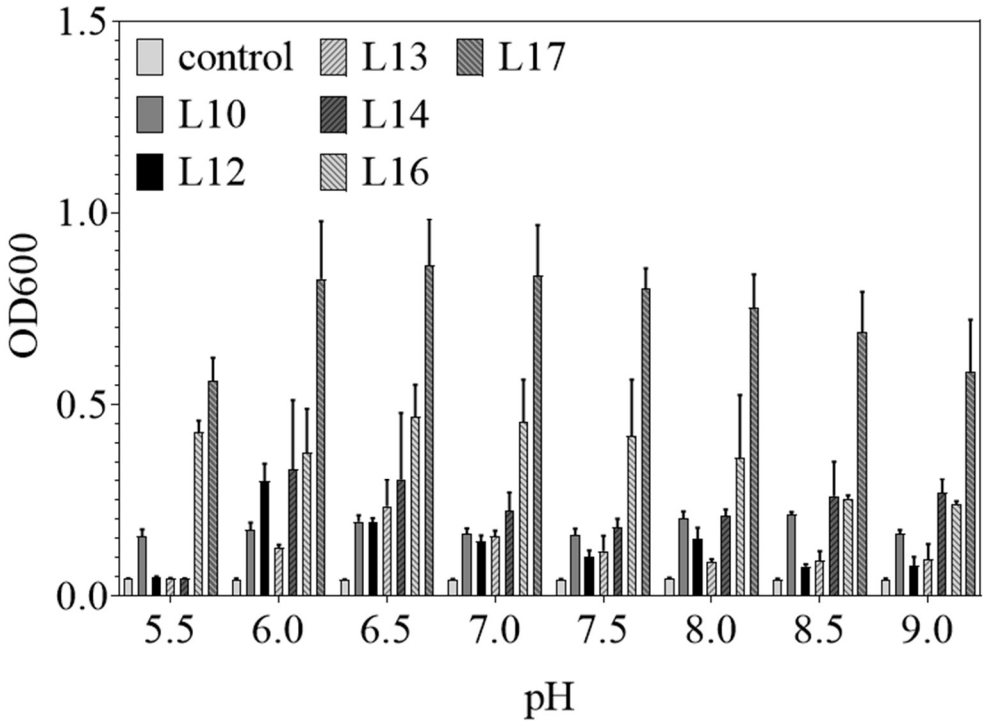
TGTTAGTCTT

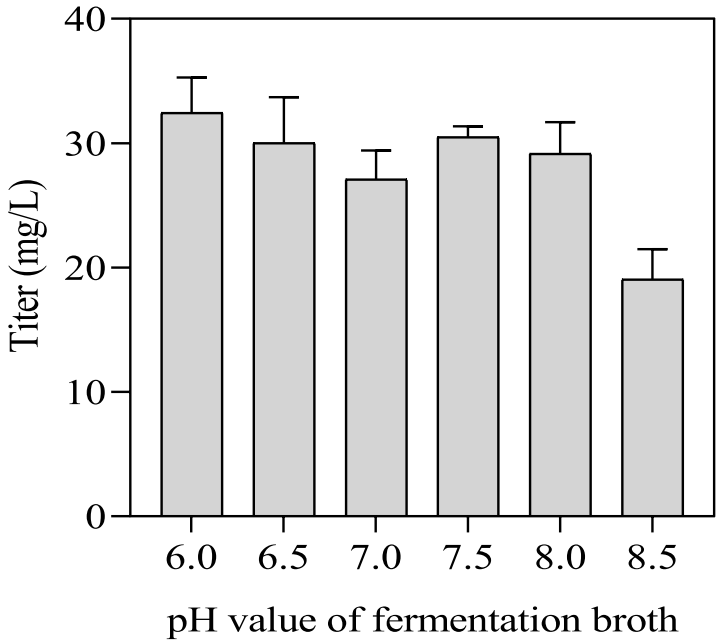
Supplementary Fig. 1 Distribution of *Tistrella* and *did* BGC in the marine biosphere. In (**a**), presence of *Tistrella* were verified using the conserved 40S ribosomal protein S3. In (**b**), cycles represent metagenomic data, and triangles represent metatranscriptomic data. Colors represent depth of sampling site below sea level.



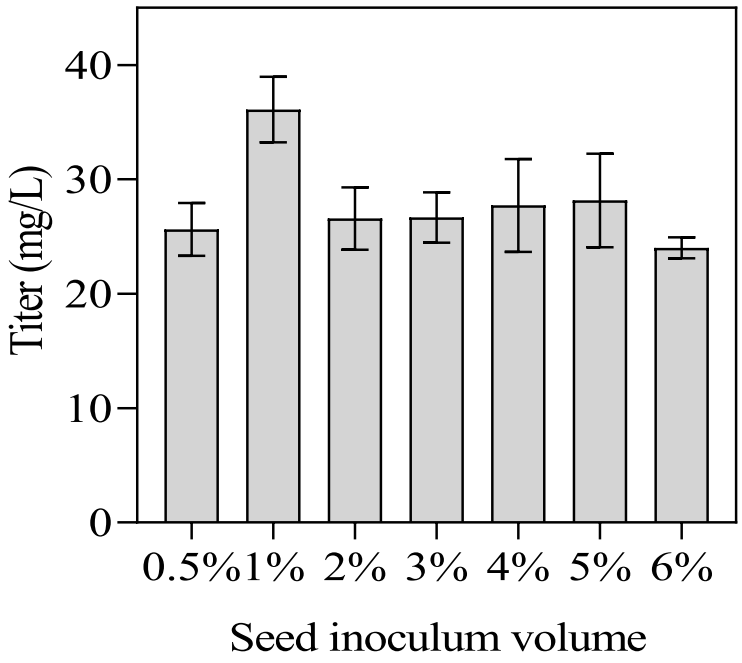
Supplementary Fig. 2 Growth state of selected *Tistrella* strains in TFM with different initial pH after 24 hours incubation.



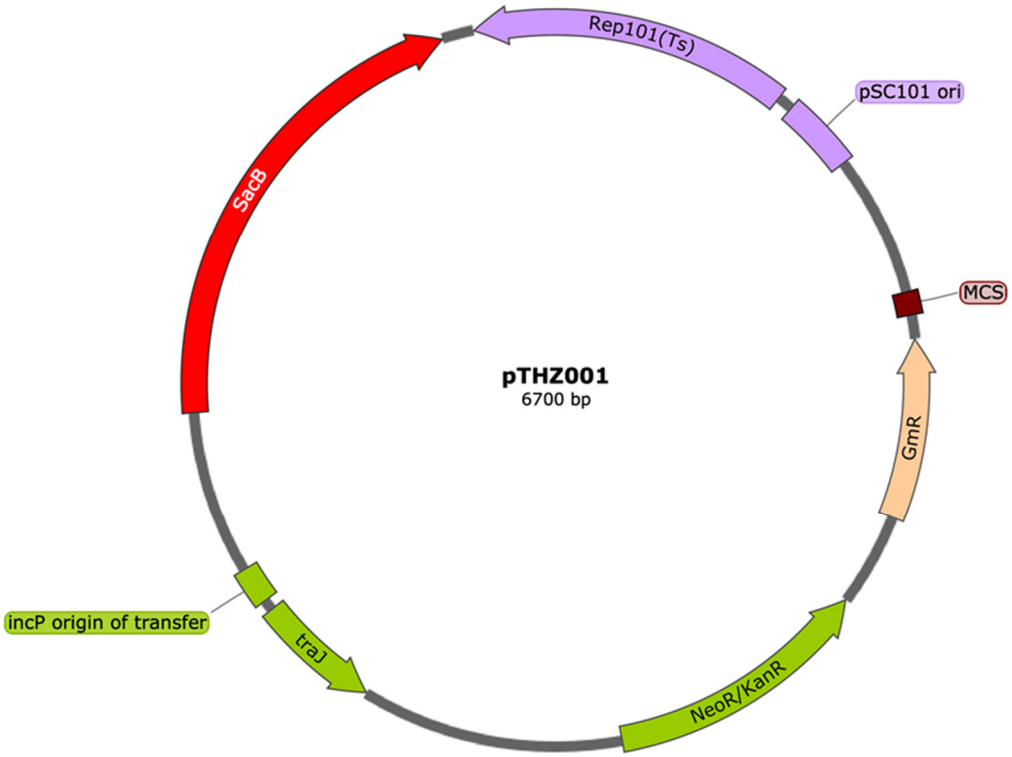
Supplementary Fig. 3 The titers of didemnin B (**2**) in the TFM with different initial pH values.



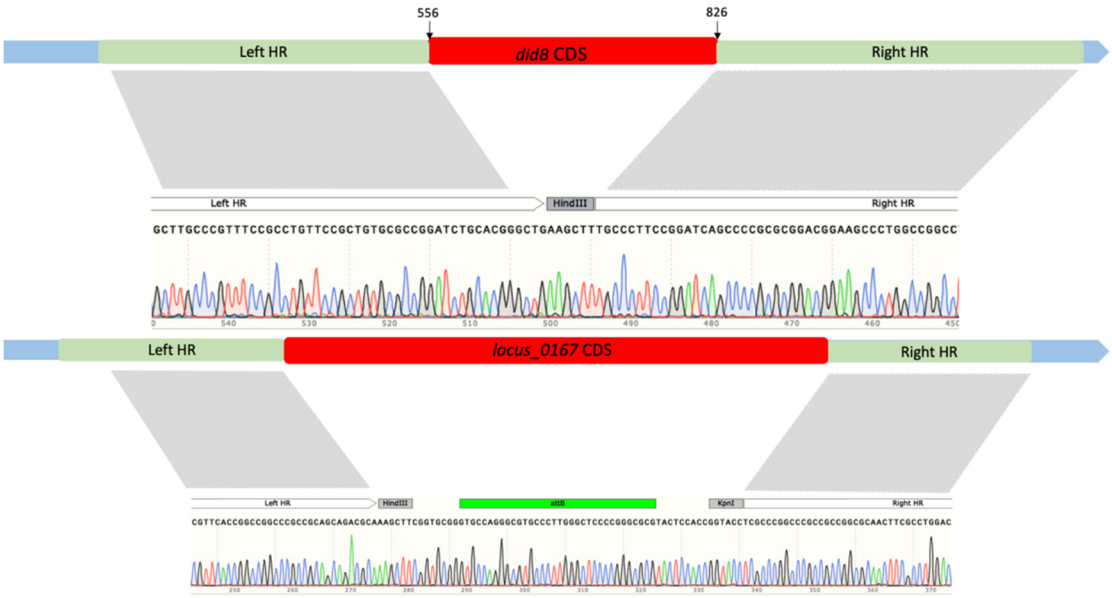
Supplementary Fig. 4 The titers of didemnin B (**2**) in the TFM with different seed volumes.



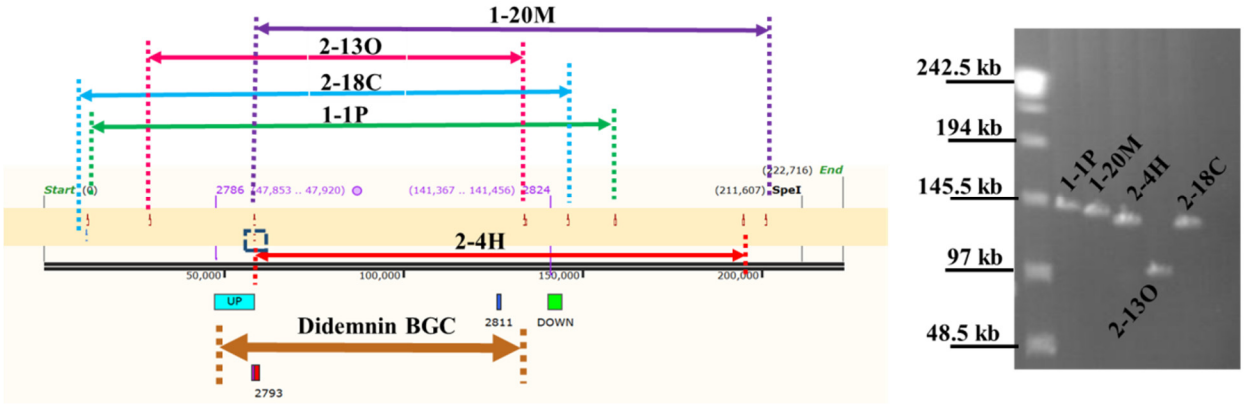
Supplementary Fig. 5 Plasmid map of pTHZ001. The vector constructed by assembling four different functional elements from pJZ001 (Tan), pJZ002 (Lavender), pCAP01 (Lime), and synthesized sacB component (Red).



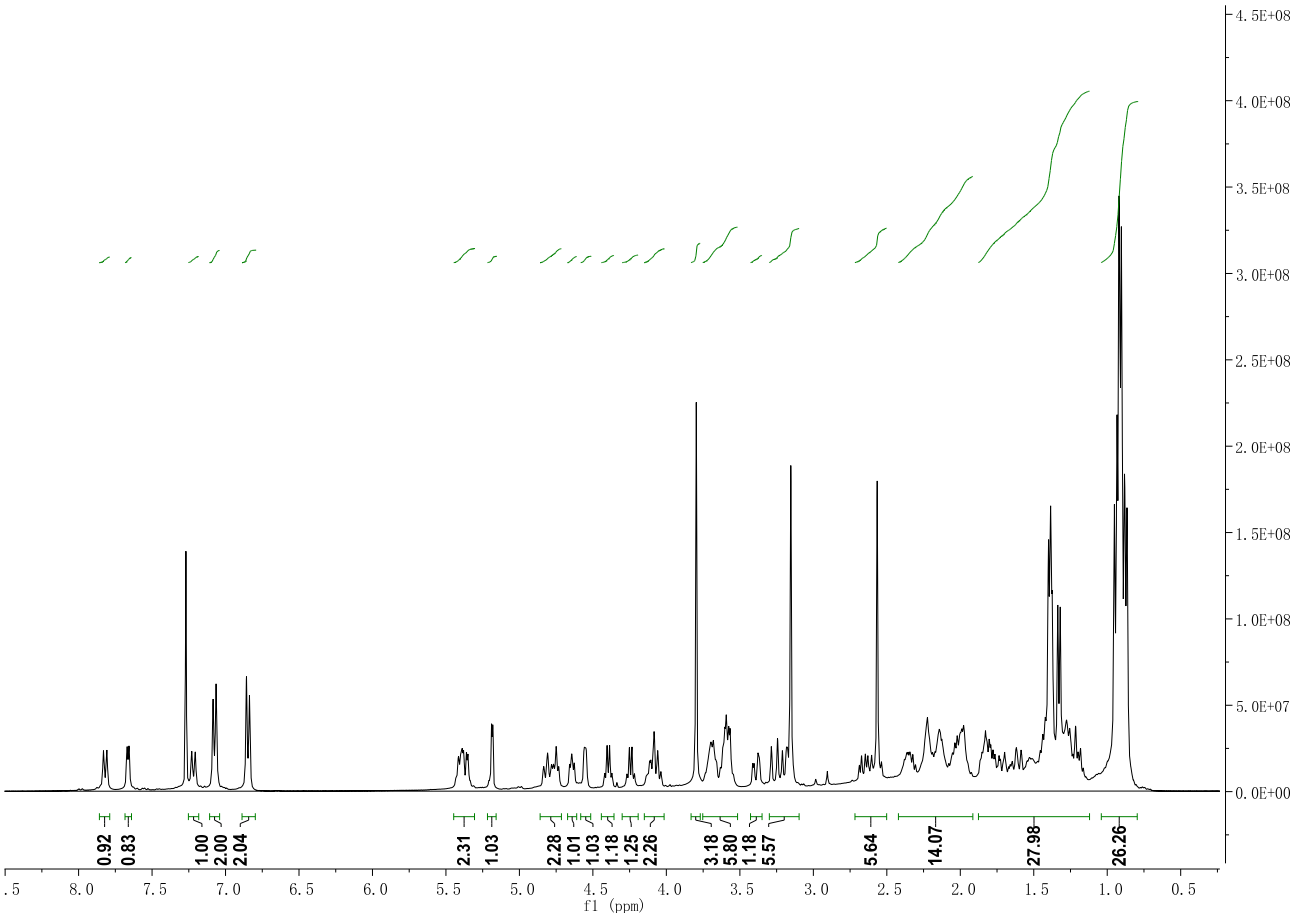
Supplementary Fig. 6 The Sanger sequencing for verifying *T. mobilis* L17/Δ*didB(556-826)* and *T. mobilis* L17::*attB* mutants. The Upper scheme shows the deletion of *didB* from the base pair number *556 to 826*. The bottom scheme reveals the deletion of locus_*0167* while insertion of ***attB*** element, which is annotated in green color in the map.



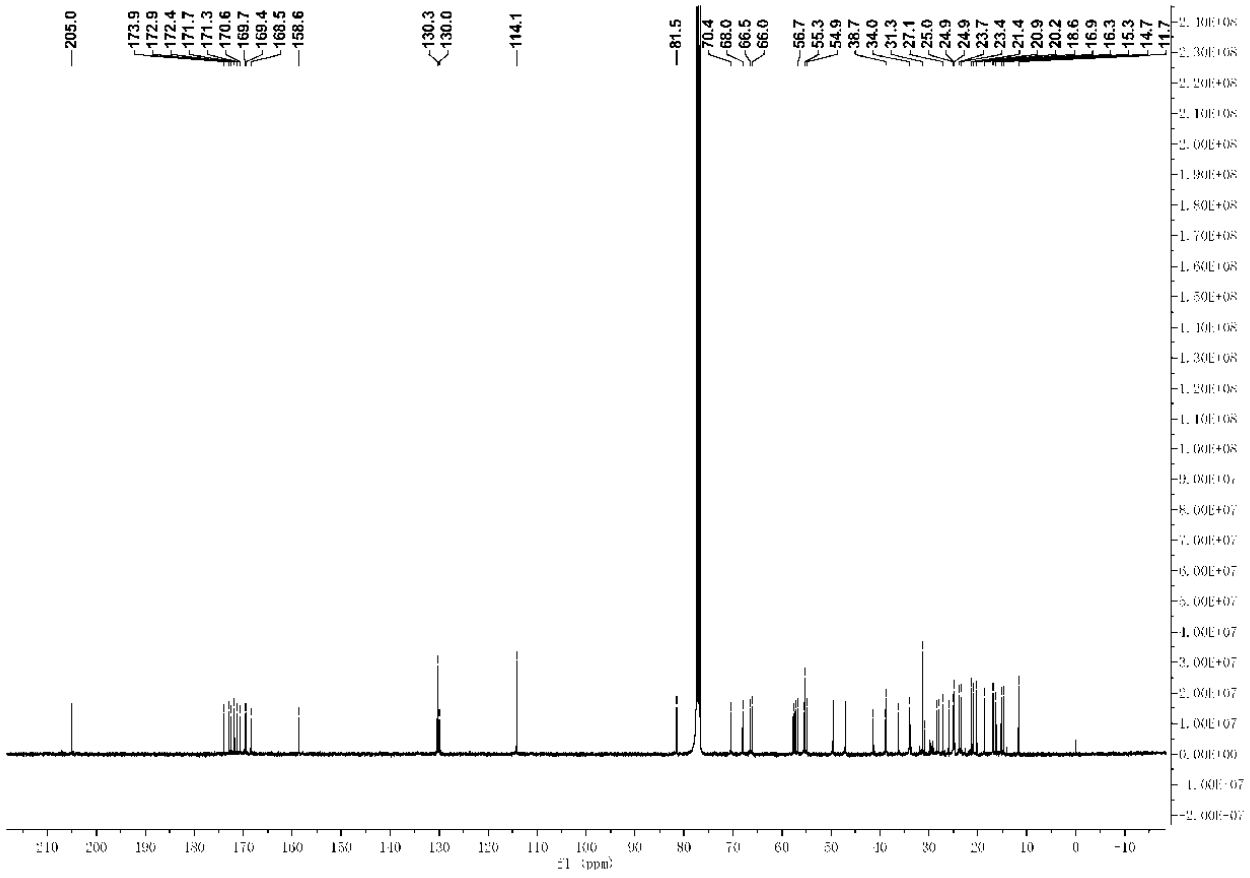
Supplementary Fig. 7 Screening of a BAC library for vectors carrying the *did* BGC.



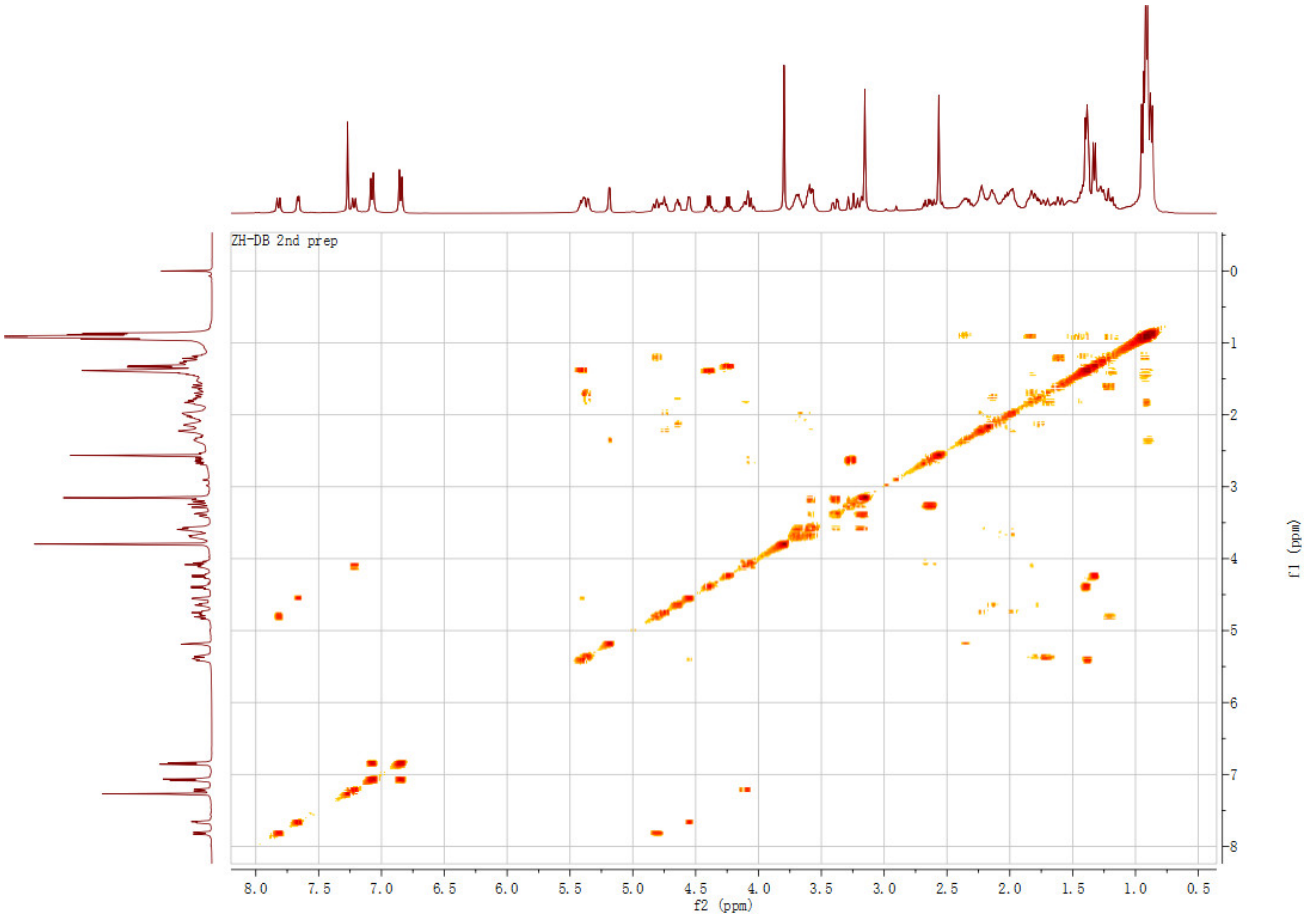
Supplementary Fig. 8 ^{1}H NMR spectrum of didemnin B (**2**, 400 MHz). Solvent: $CDCl_3$.



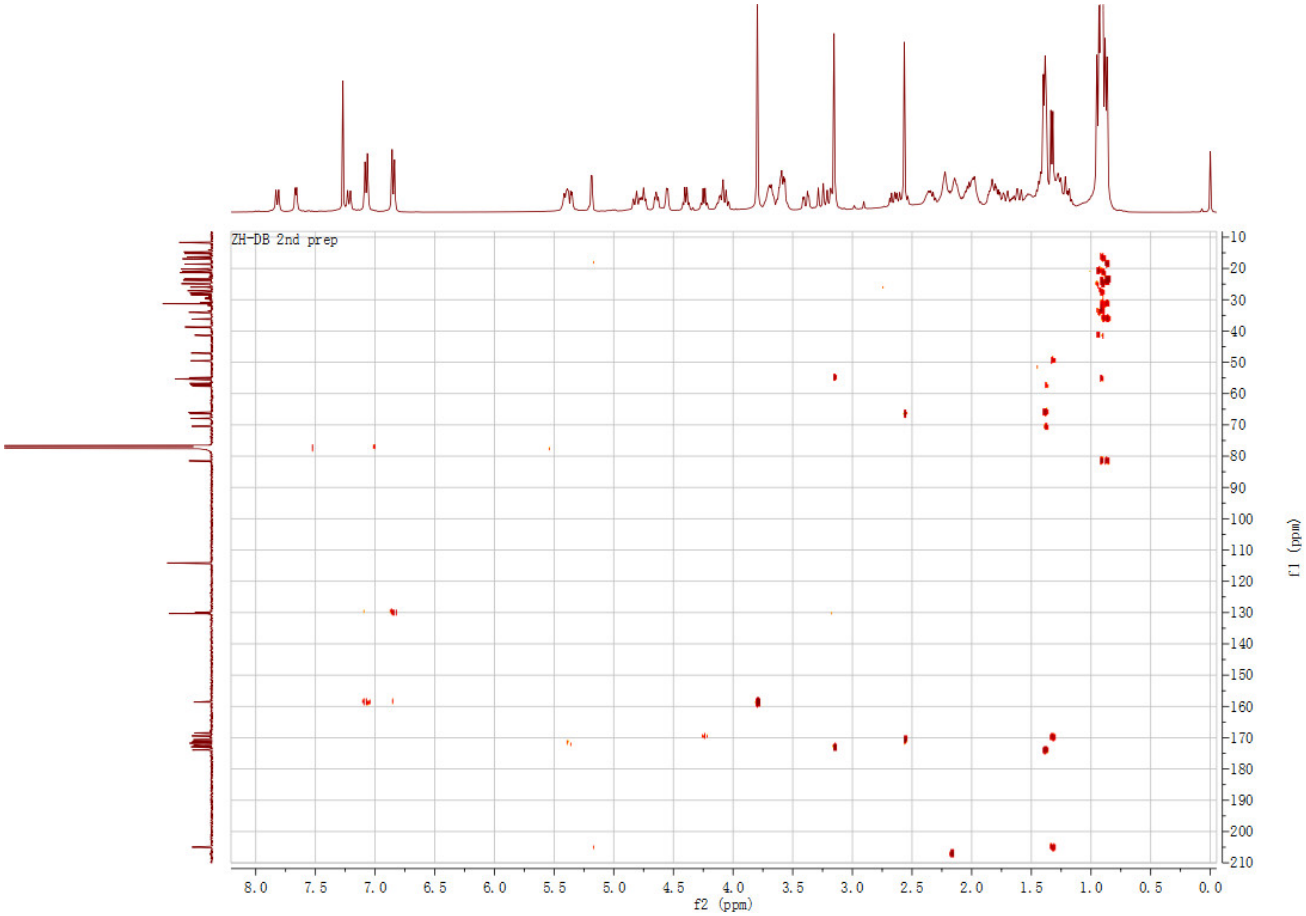
Supplementary Fig. 9 ^{13}C NMR spectrum of didemnin B (**2**, 100 MHz). Solvent: $CDCl_3$.



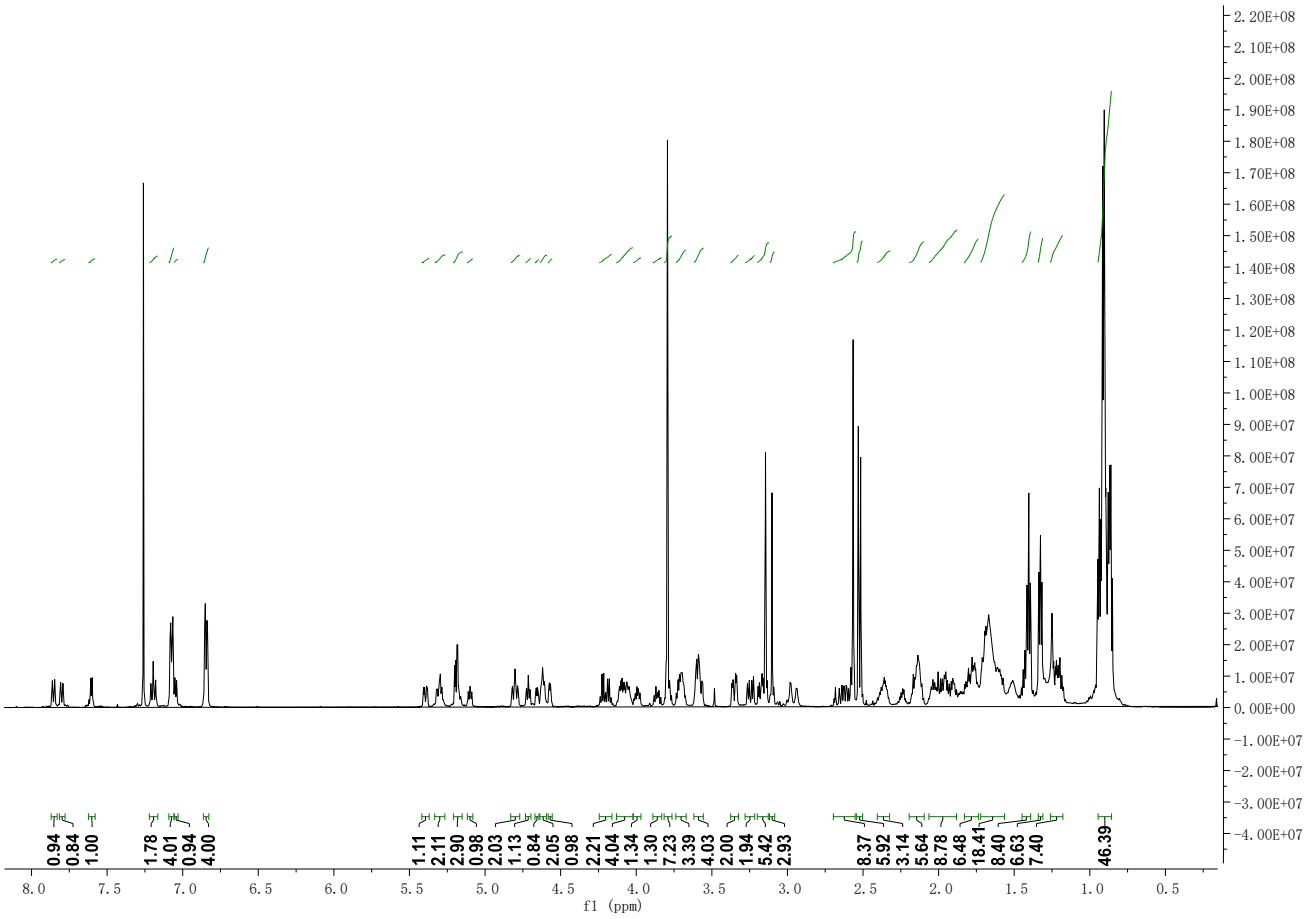
Supplementary Fig. 10 ^{1}H-^{1}H COSY of didemnin B (**2**). Solvent: $CDCl_3$.



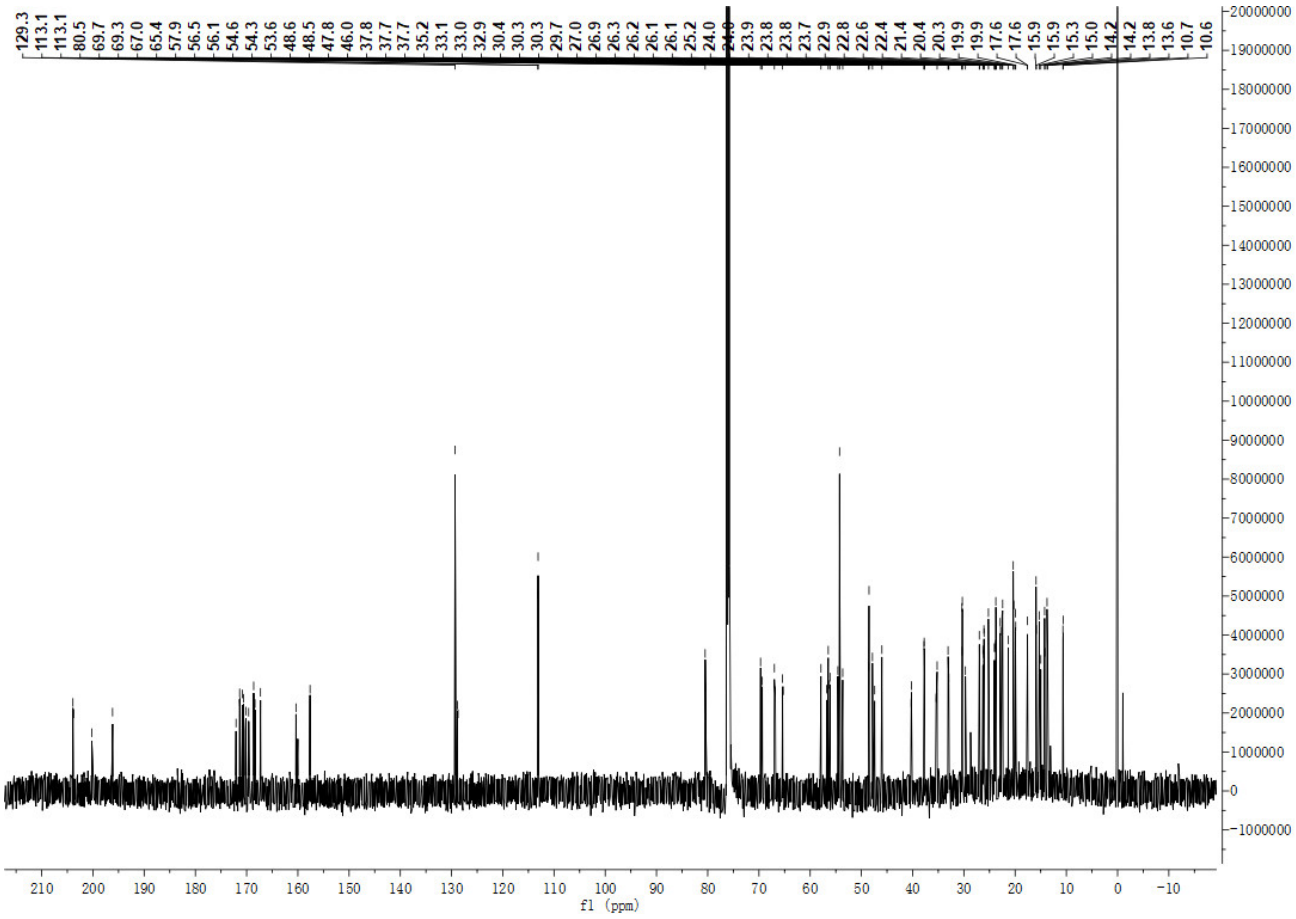
Supplementary Fig. 11 ^{1}H-^{13}C-HMBC NMR spectrum of didemnin B (**2**). Solvent: $CDCl_3$.



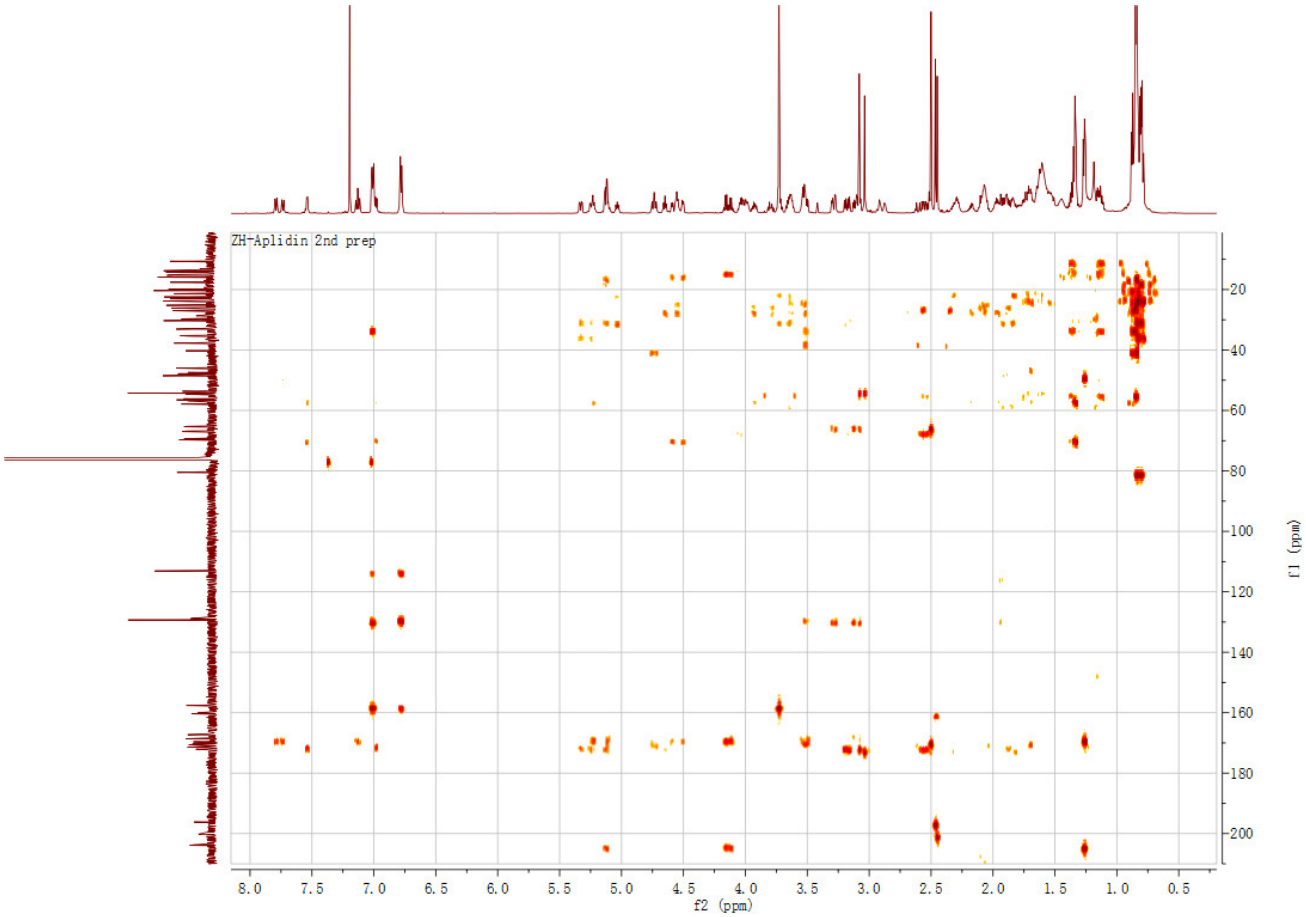
Supplementary Fig. 12 ^{1}H NMR spectrum of plitidepsin (**1**, 600 MHz). Solvent: $CDCl_3$.



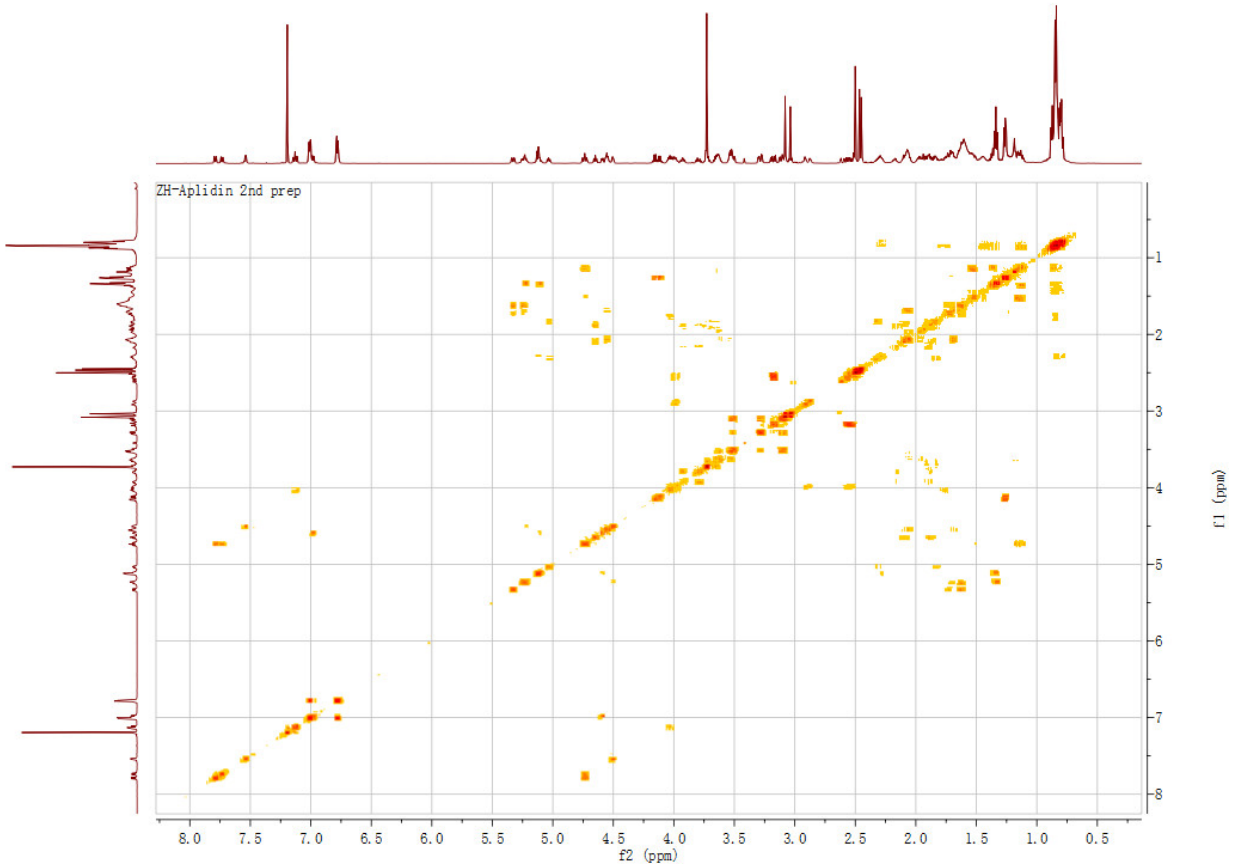
Supplementary Fig. 13 ^{13}C NMR spectrum of plitidepsin (**1**, 125 MHz). Solvent: $CDCl_3$.



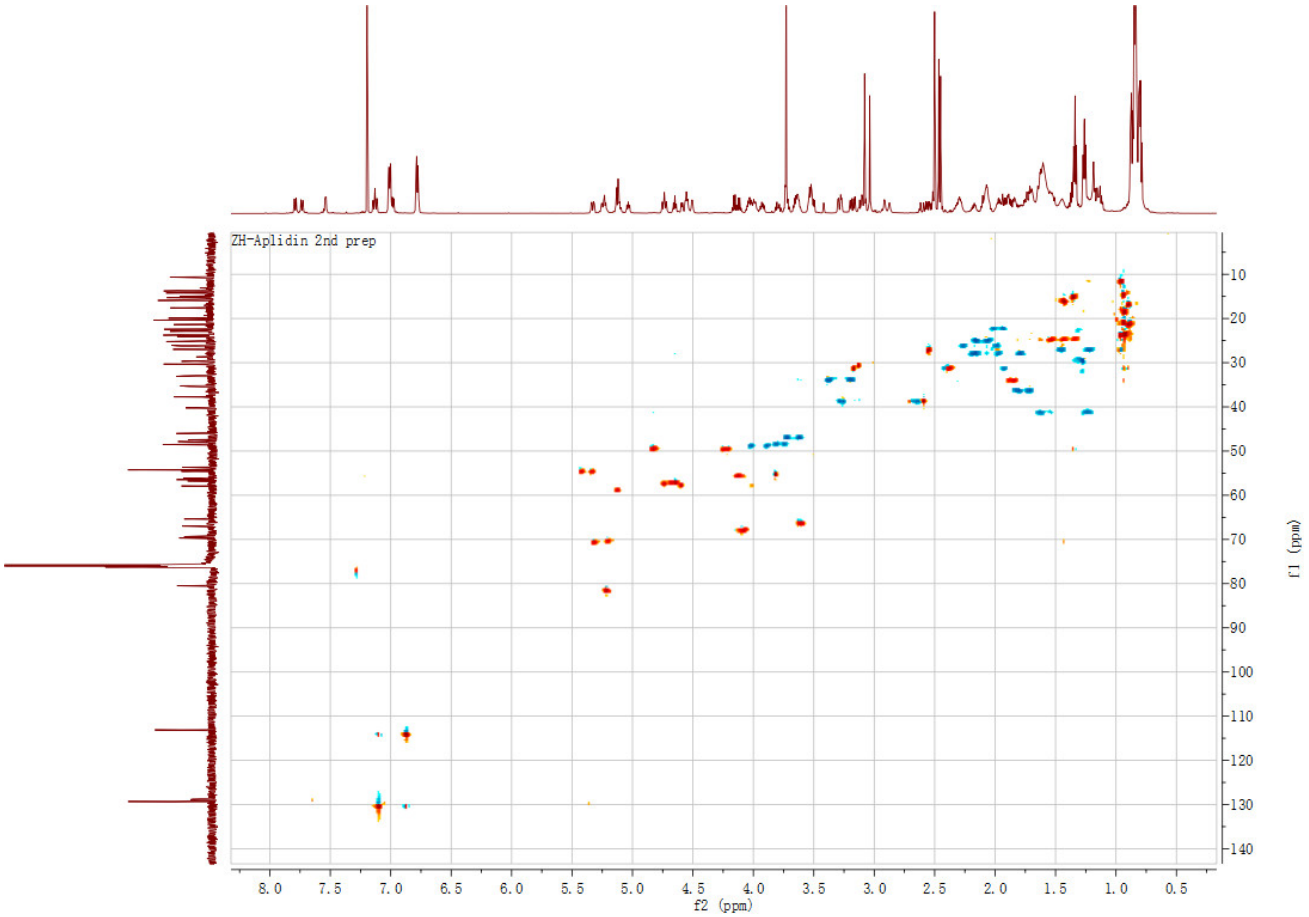
Supplementary Fig. 14 ^{1}H-^{13}C-HMBC of plitidepsin (**1**). Solvent: $CDCl_3$.



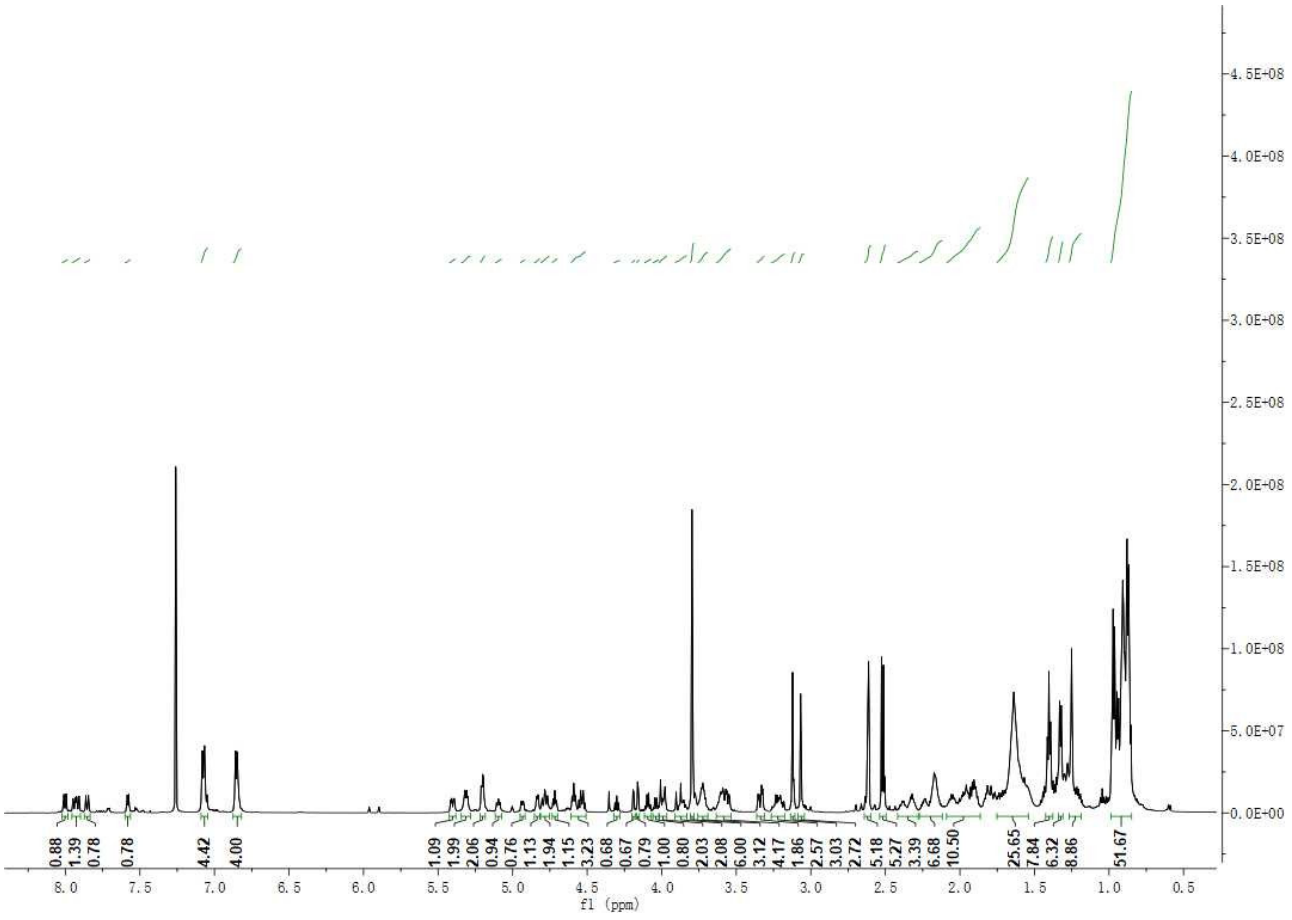
Supplementary Fig. 15 ^{1}H-^{1}H COSY of plitidepsin (**1**). Solvent: $CDCl_3$.



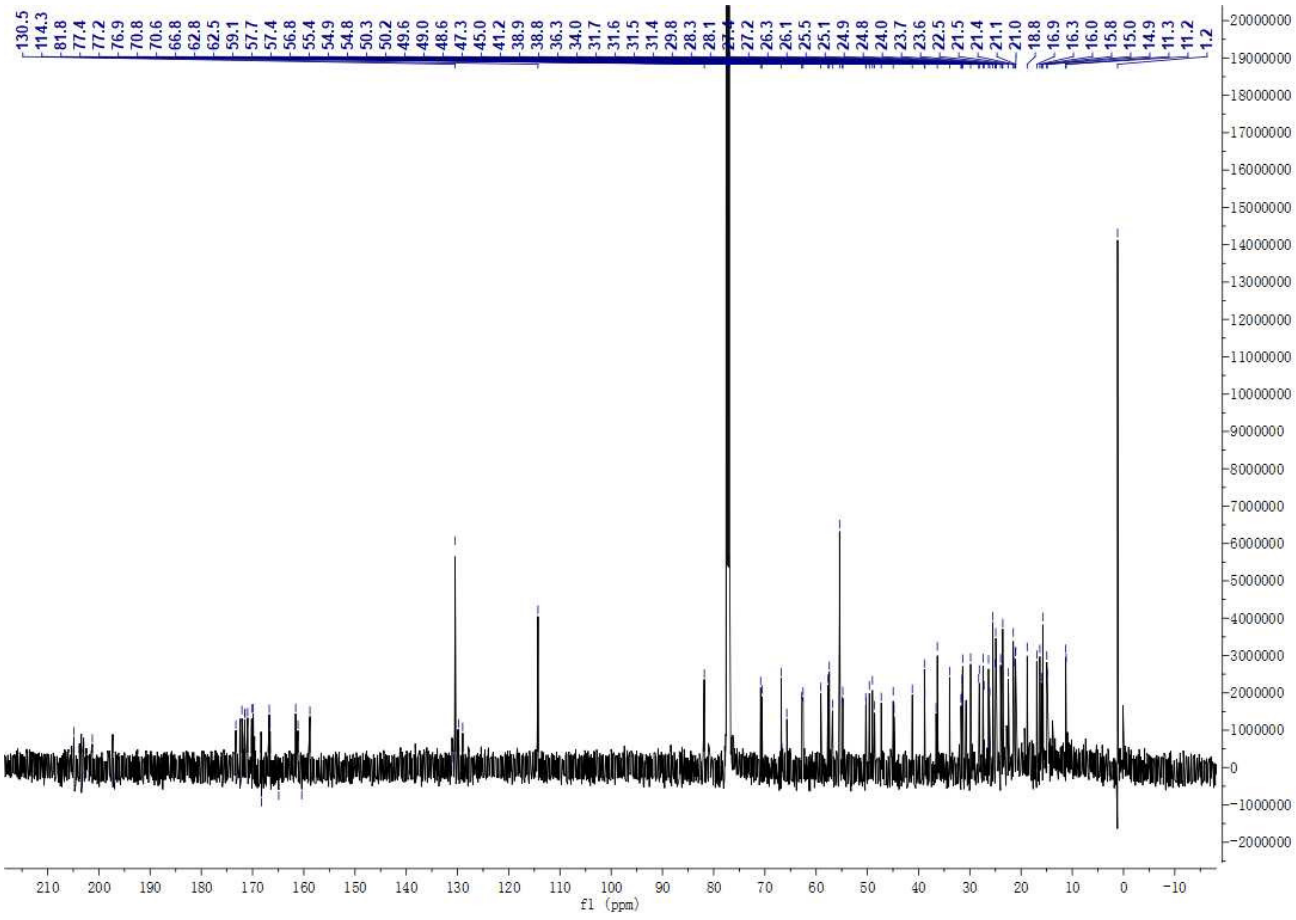
Supplementary Fig. 16 ^{1}H-^{13}C HSQC of plitidepsin (**1**). Solvent: $CDCl_3$.



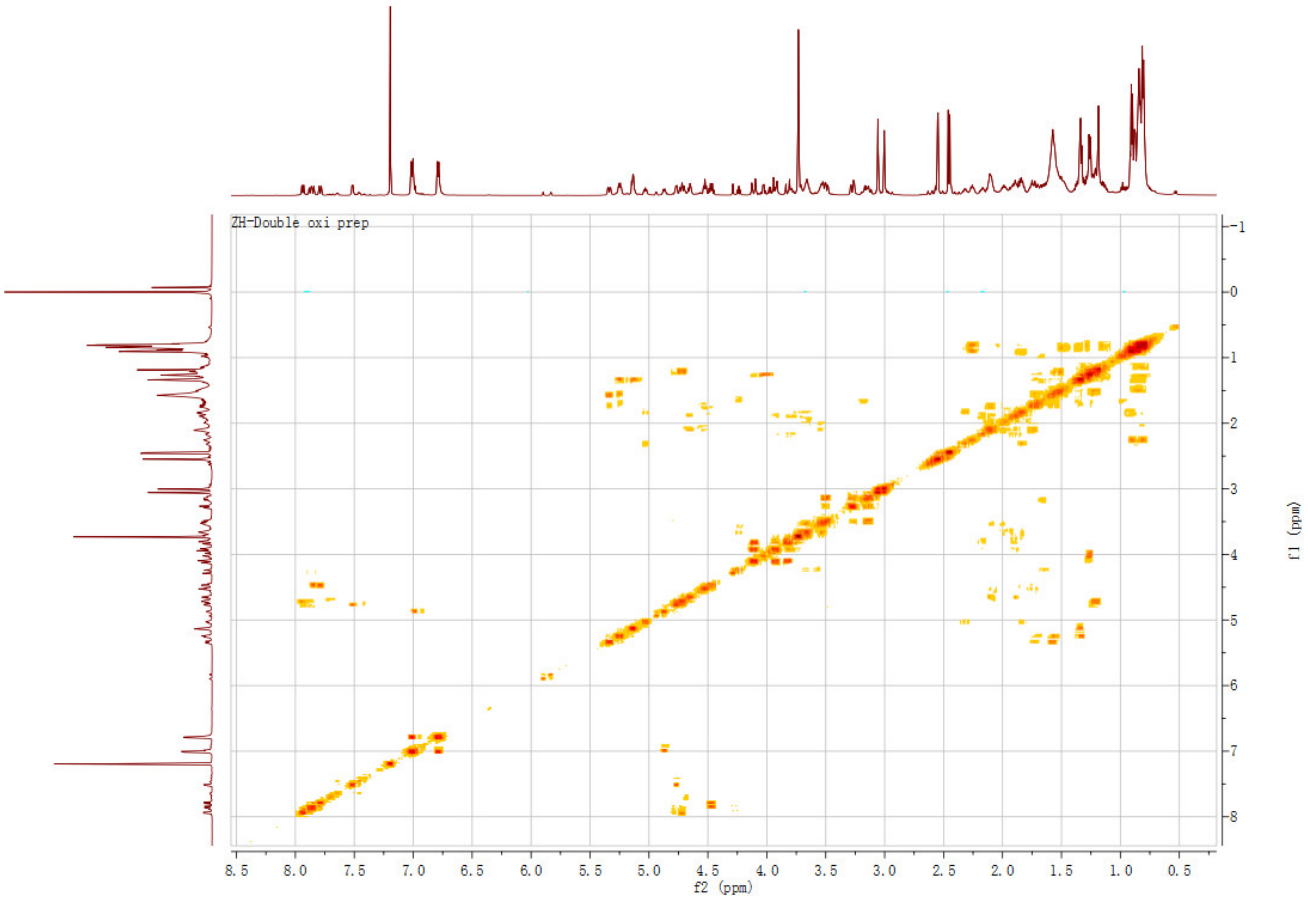
Supplementary Fig. 17 ^{1}H NMR of compound **5** (600 MHz). Solvent: $CDCl_3$.



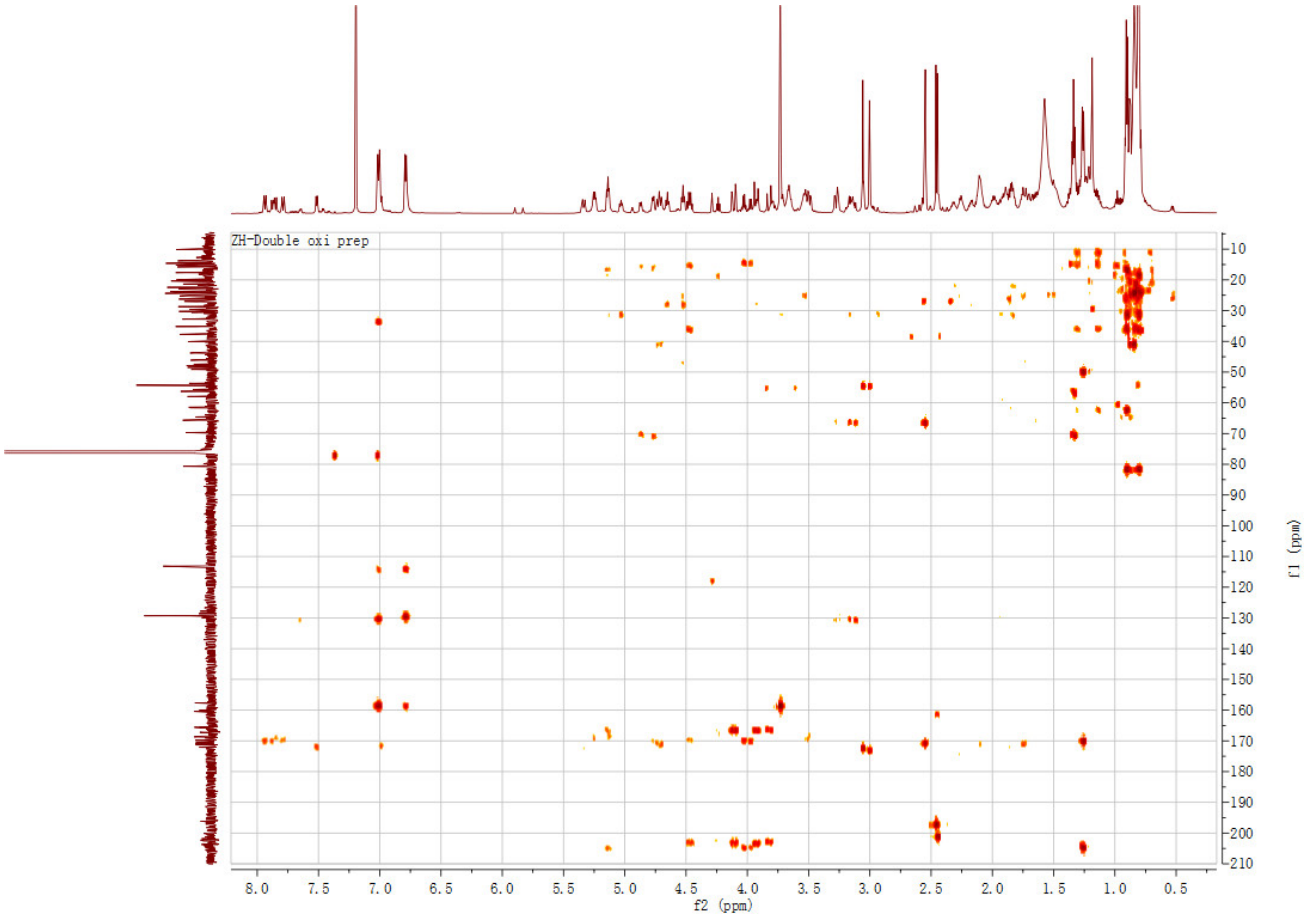
Supplementary Fig. 18 ^{13}C NMR of compound **5** (125 MHz). Solvent: $CDCl_3$.



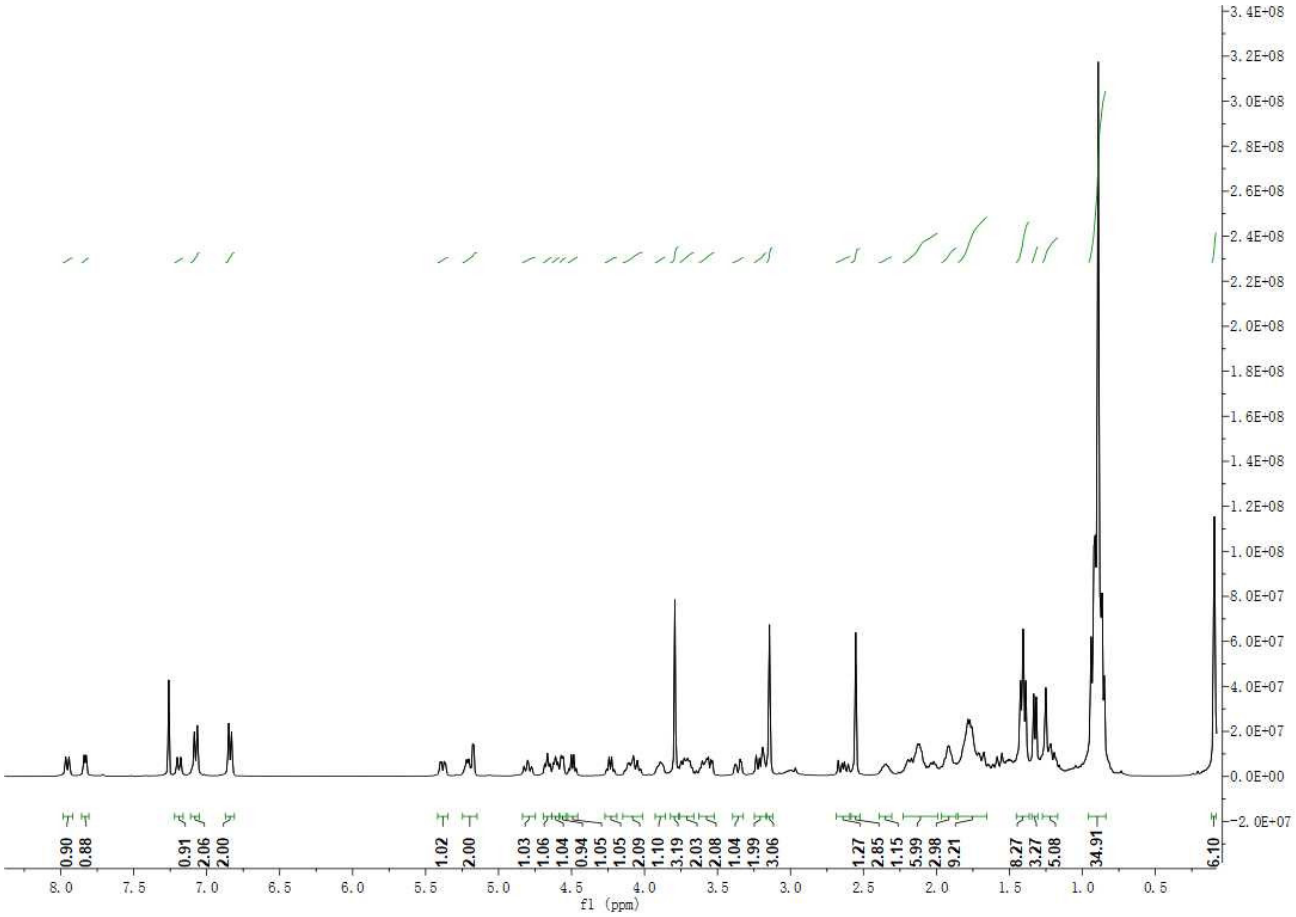
Supplementary Fig. 19 ^{1}H-^{1}H COSY of compound **5**. Solvent: $CDCl_3$.



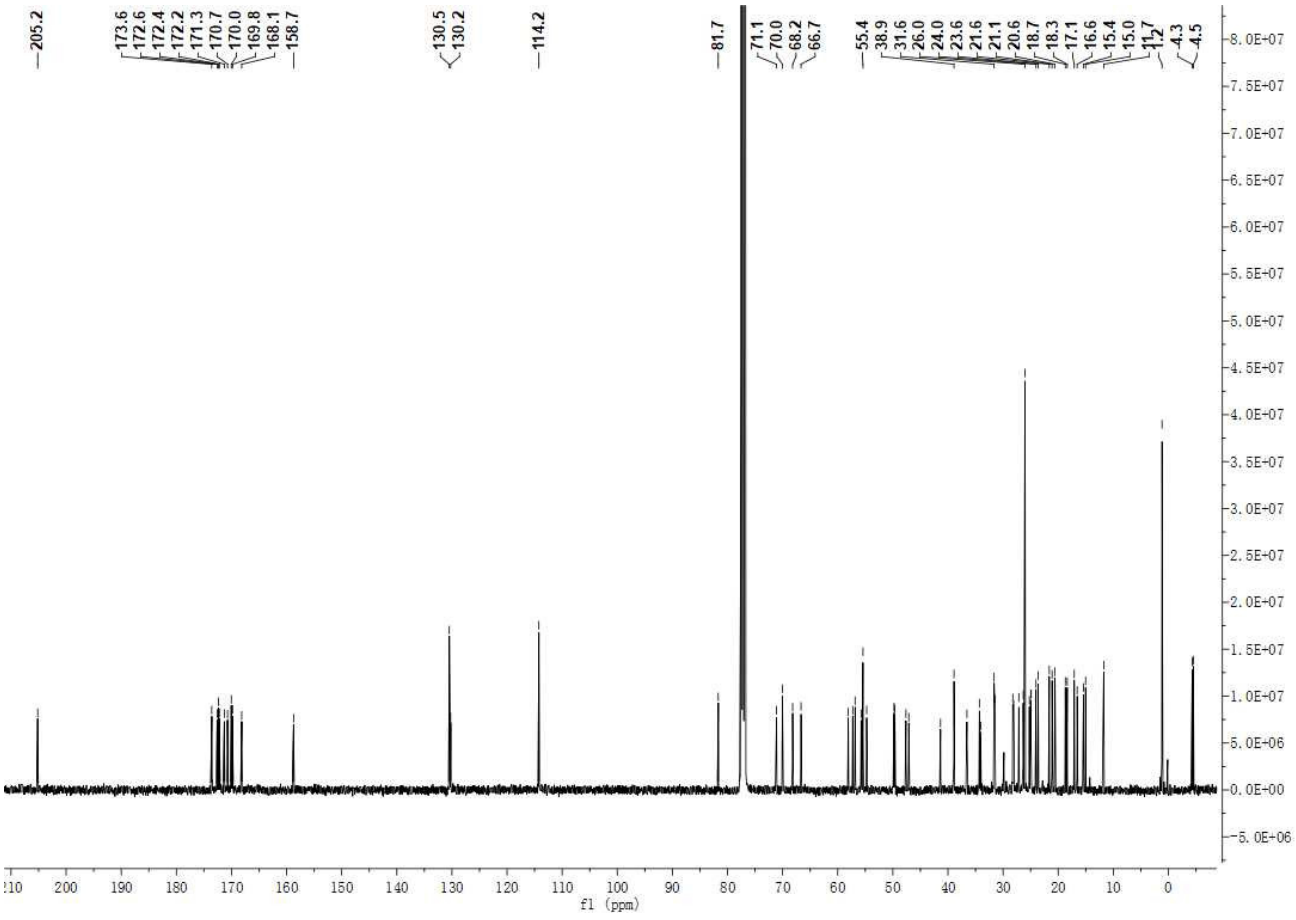
Supplementary Fig. 20 ^{1}H-^{13}C HMBC of compound **5**. Solvent: $CDCl_3$.



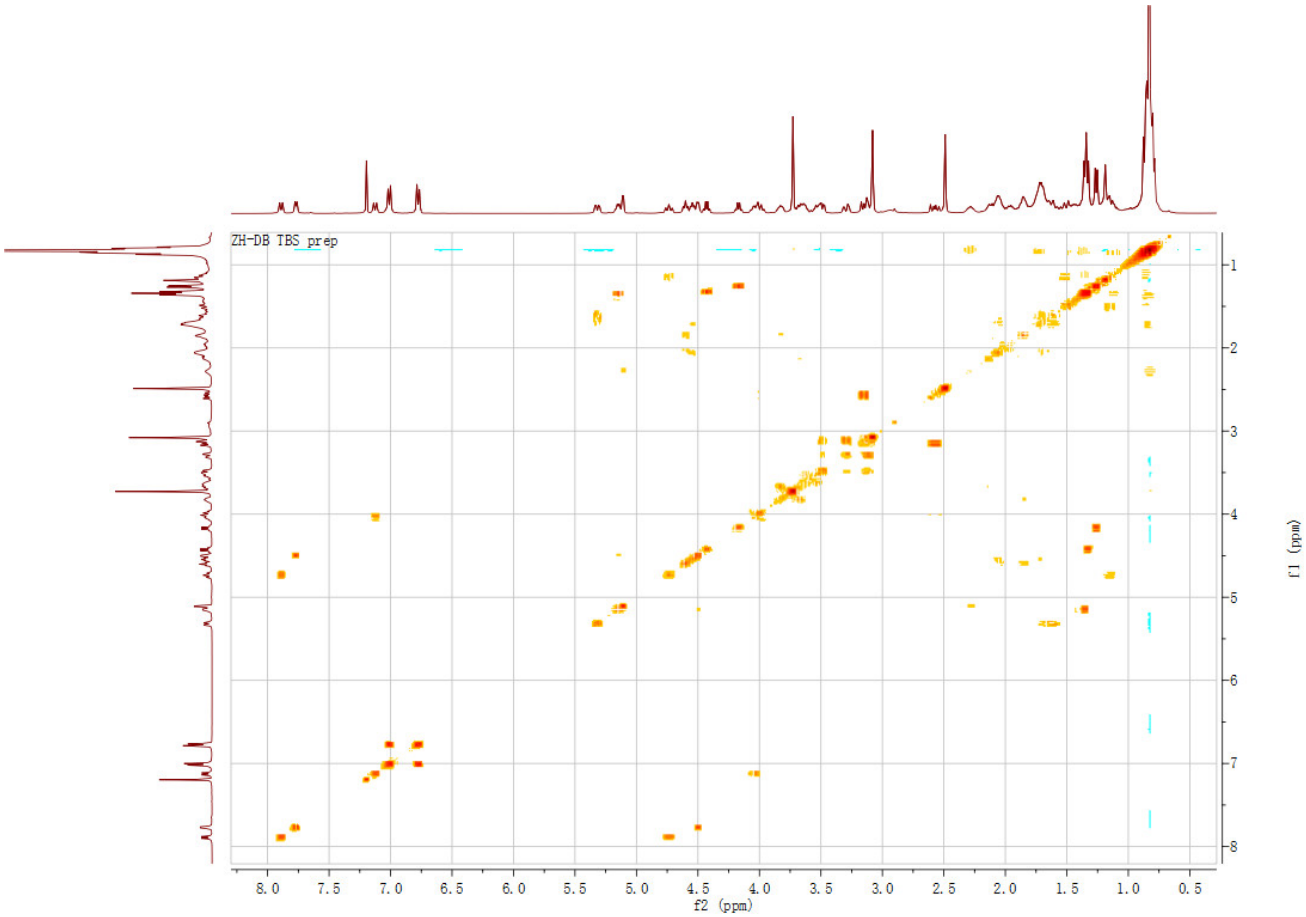
Supplementary Fig. 21 ^{1}H NMR of compound **7** (600 MHz). Solvent: $CDCl_3$.



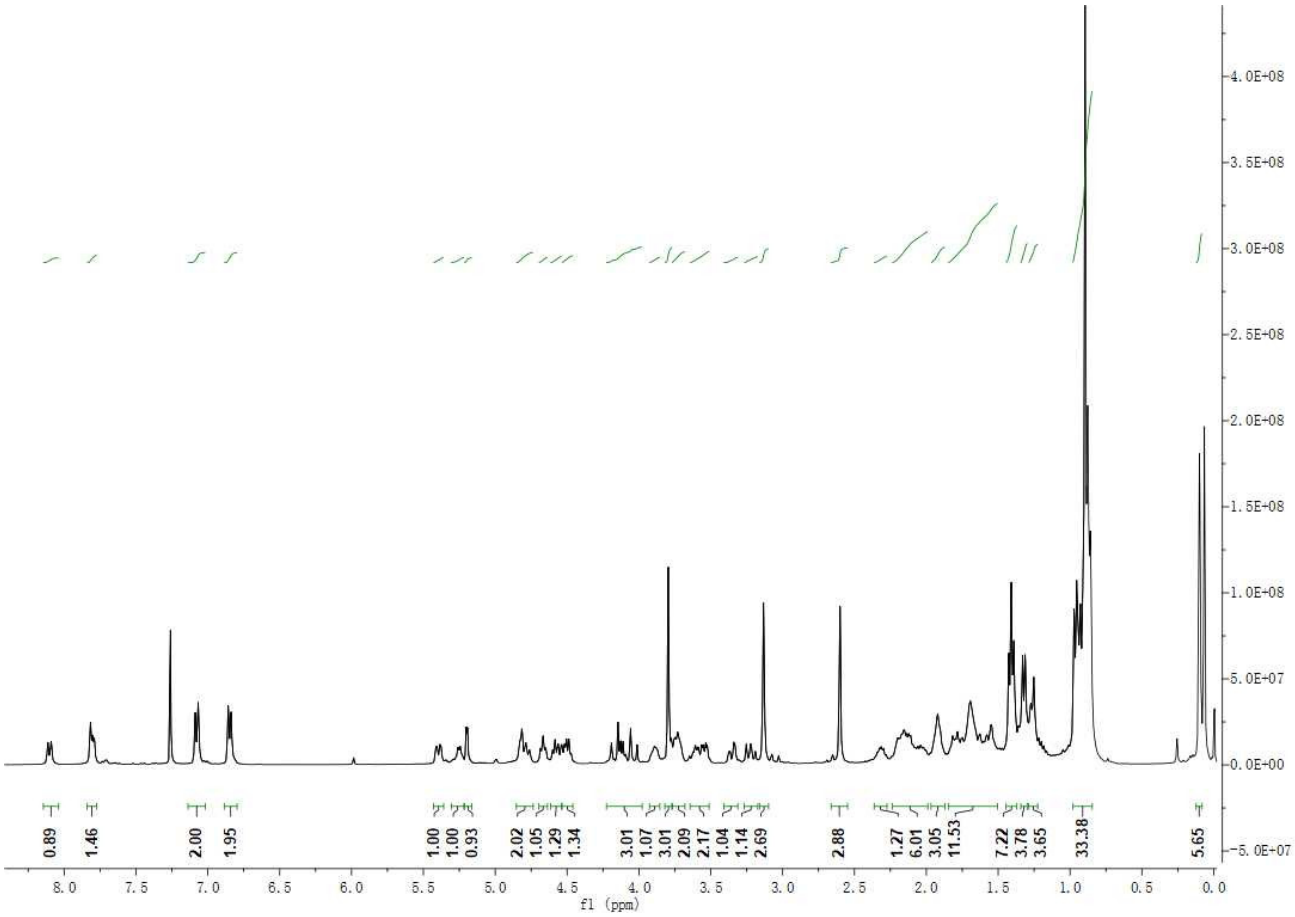
Supplementary Fig. 22 ^{13}C NMR of compound **7** (125 MHz). Solvent: $CDCl_3$.



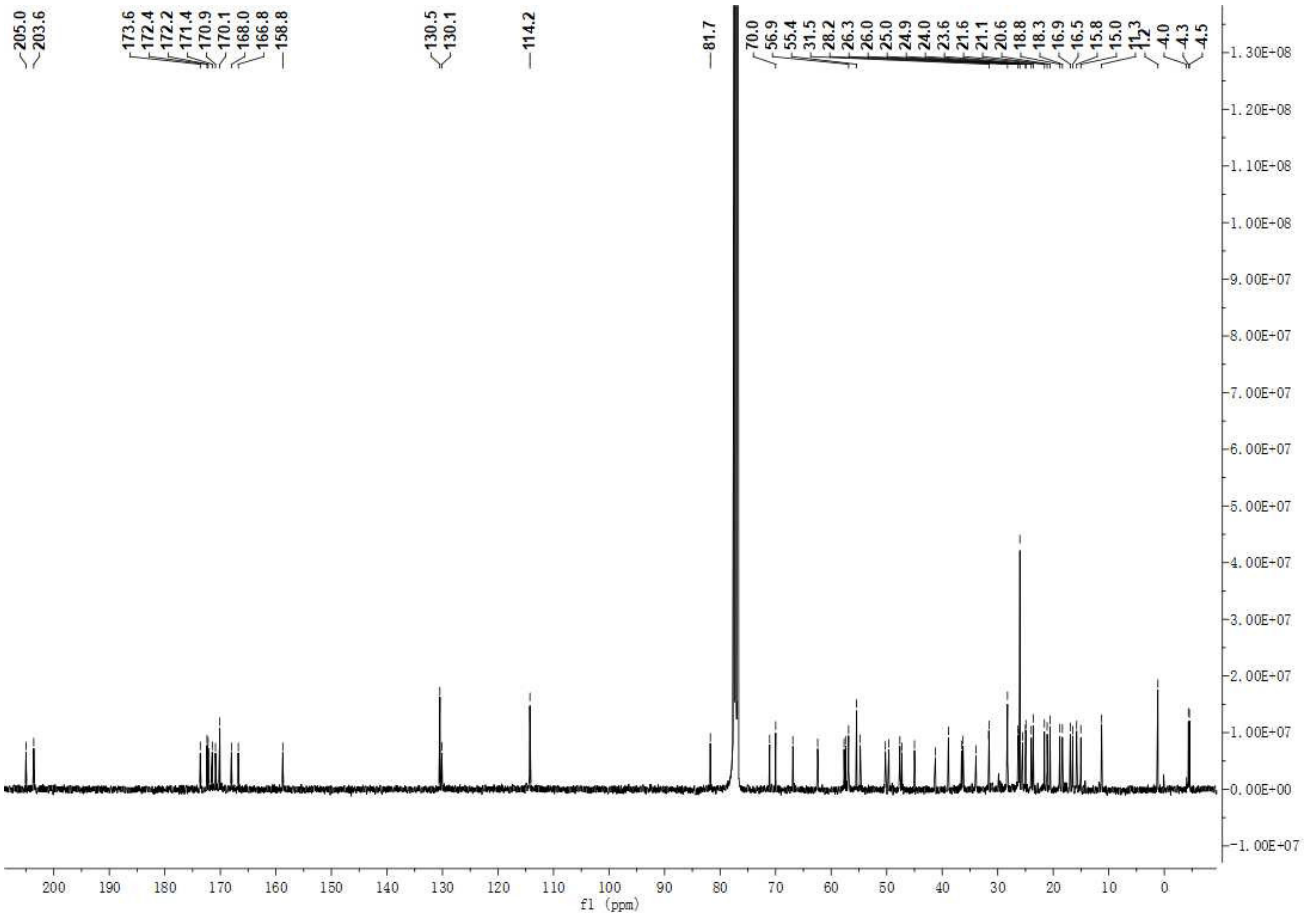
Supplementary Fig. 23 ^{1}H-^{1}H COSY of compound **7** (600 MHz). Solvent: $CDCl_3$.



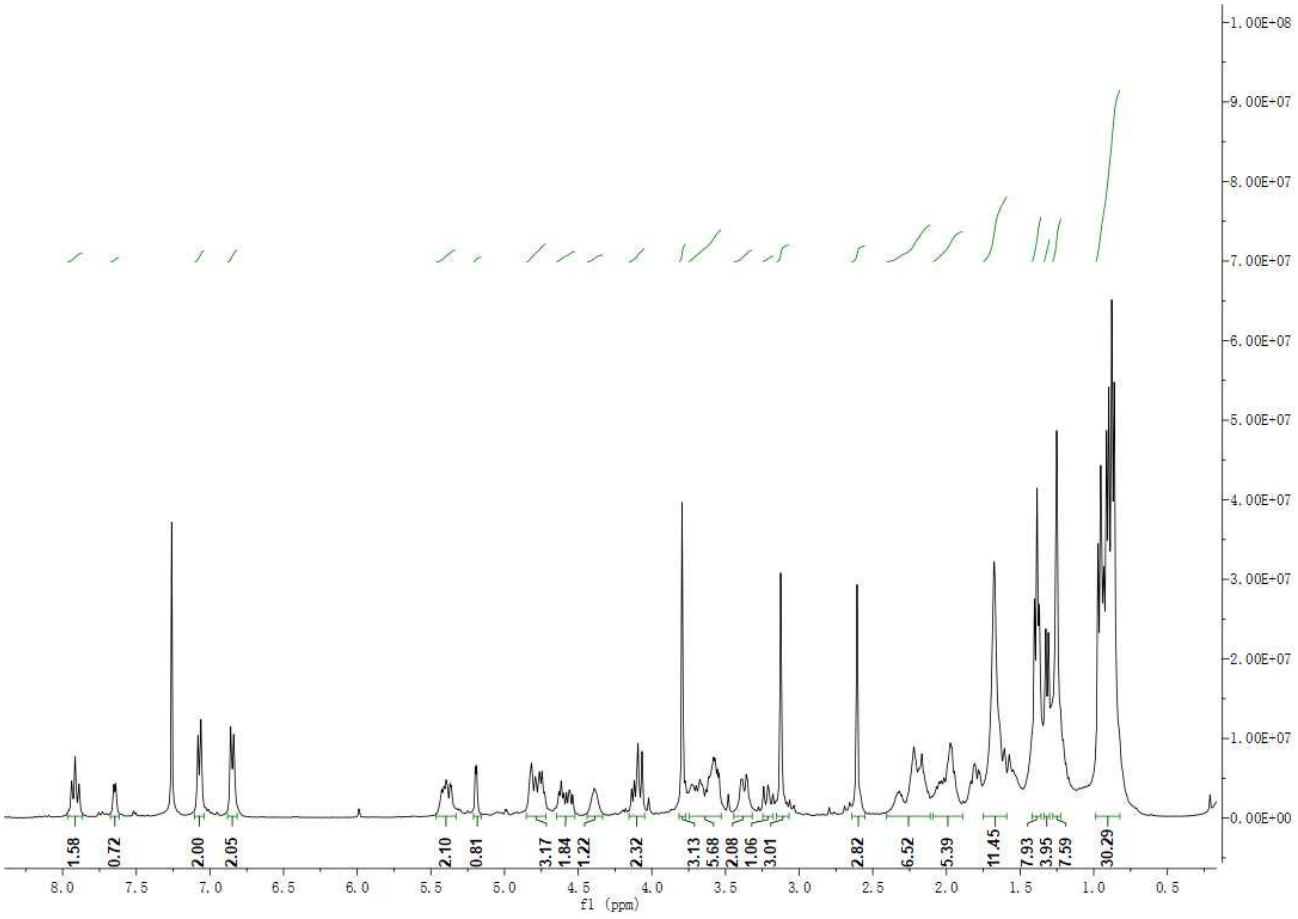
Supplementary Fig. 24 ^{1}H NMR of compound **8** (600 MHz). Solvent: $CDCl_3$.



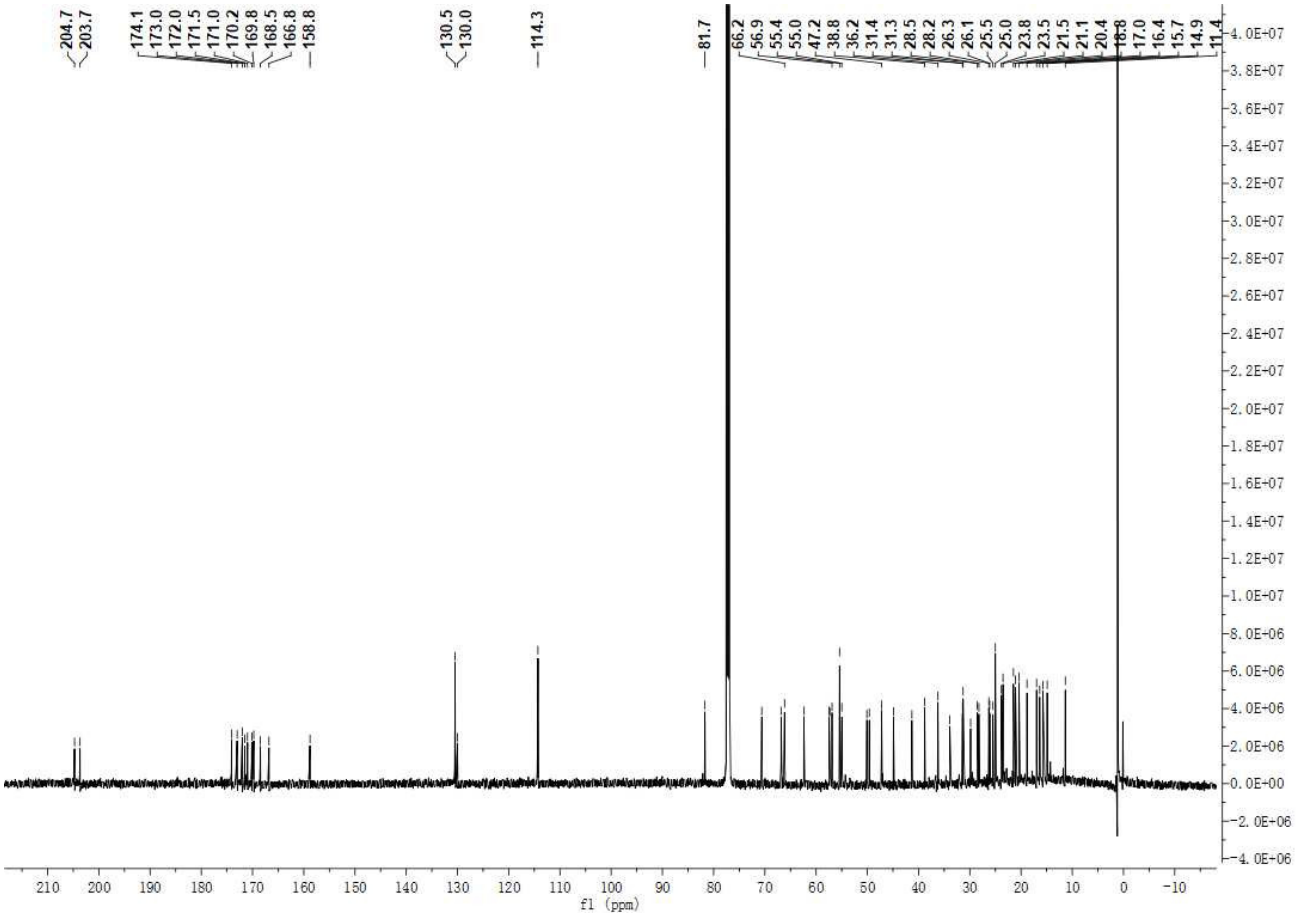
Supplementary Fig. 25 ^{13}C NMR of compound **8** (125 MHz). Solvent: $CDCl_3$.



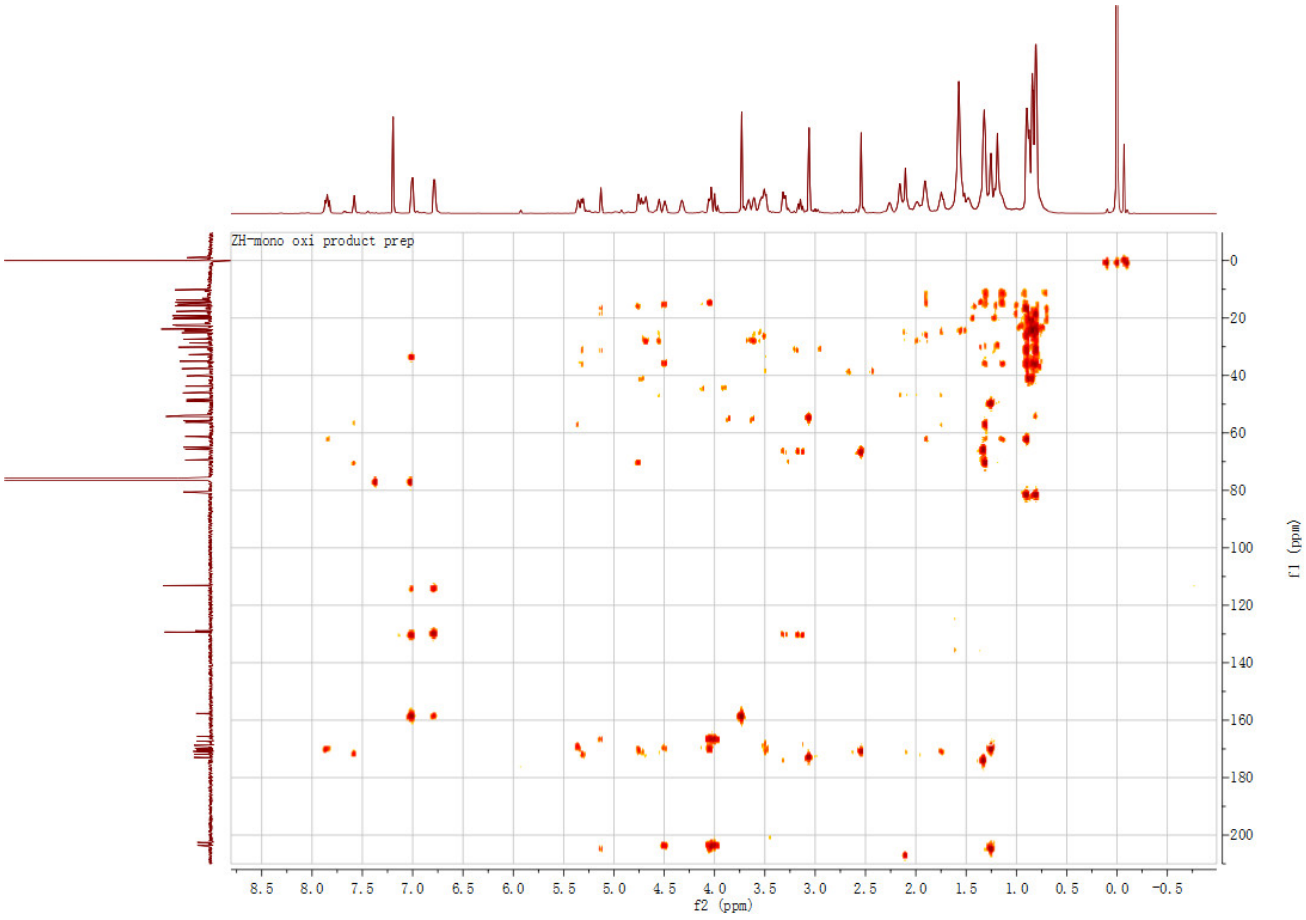
Supplementary Fig. 26 ^{1}H NMR spectrum of compound **6** (400 MHz). Solvent: $CDCl_3$.



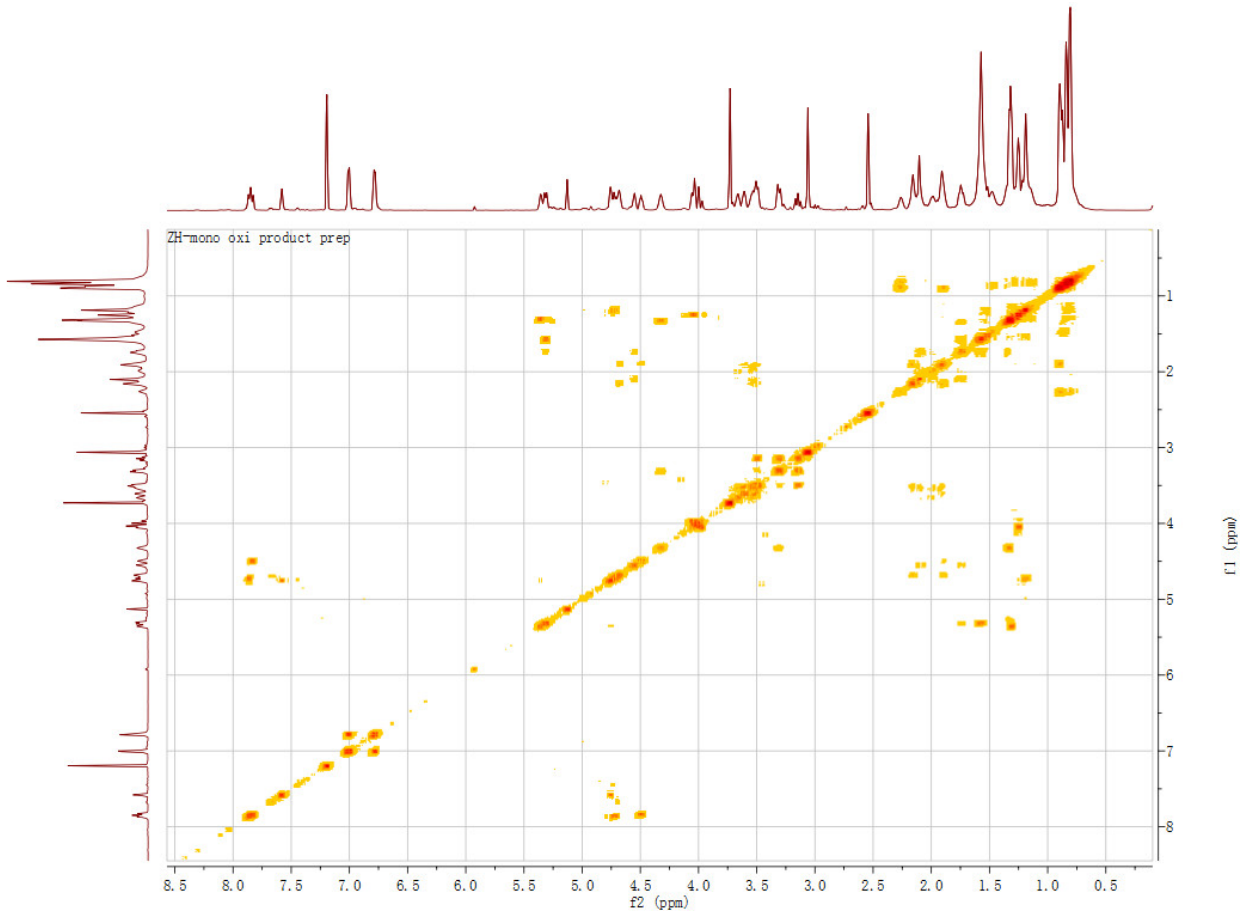
Supplementary Fig. 27 ^{13}C NMR spectrum of compound **6** (100 MHz). Solvent: $CDCl_3$.



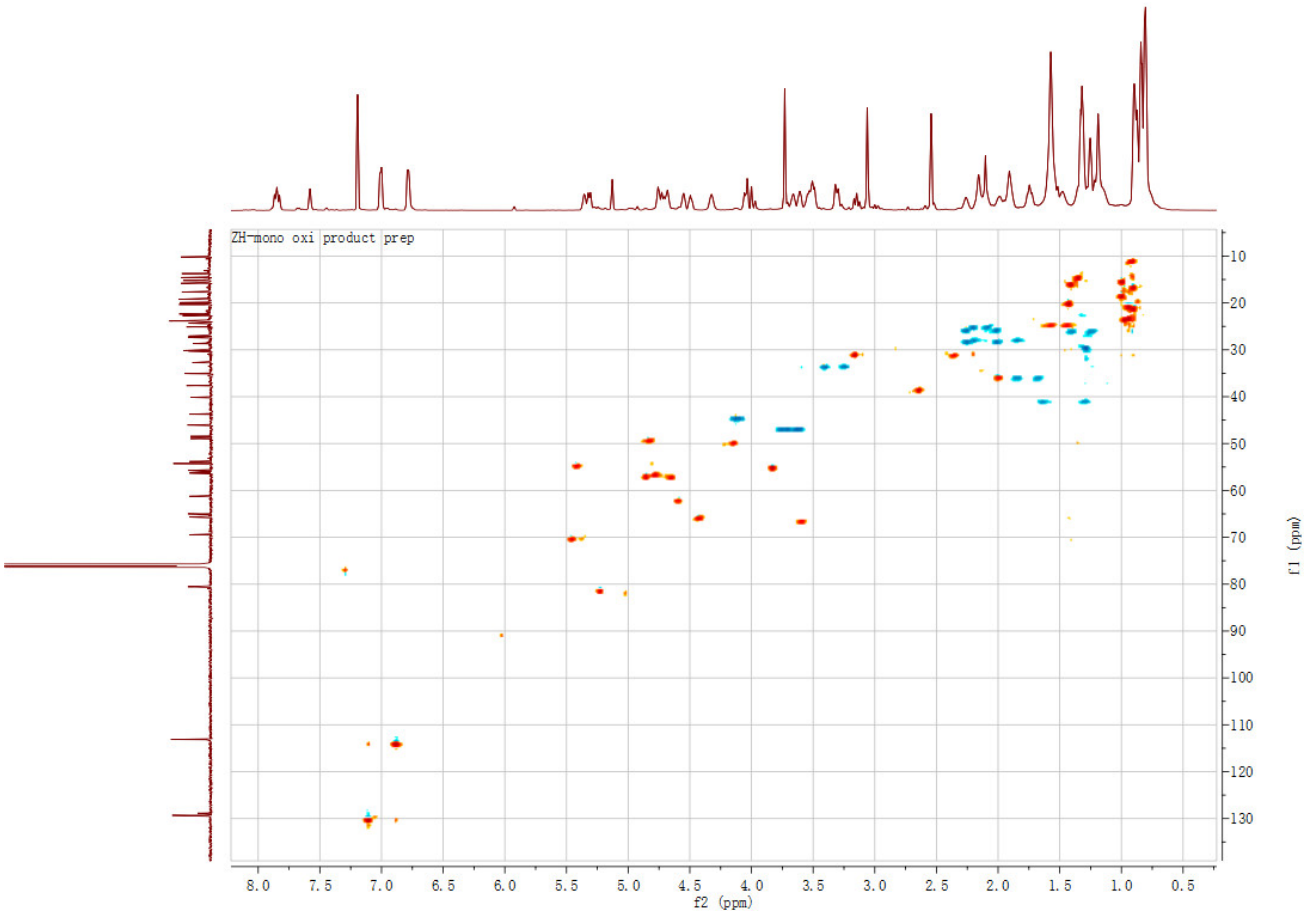
Supplementary Fig. 28 ^{1}H-^{13}C HMBC spectrum of compound **6**. Solvent: $CDCl_3$.



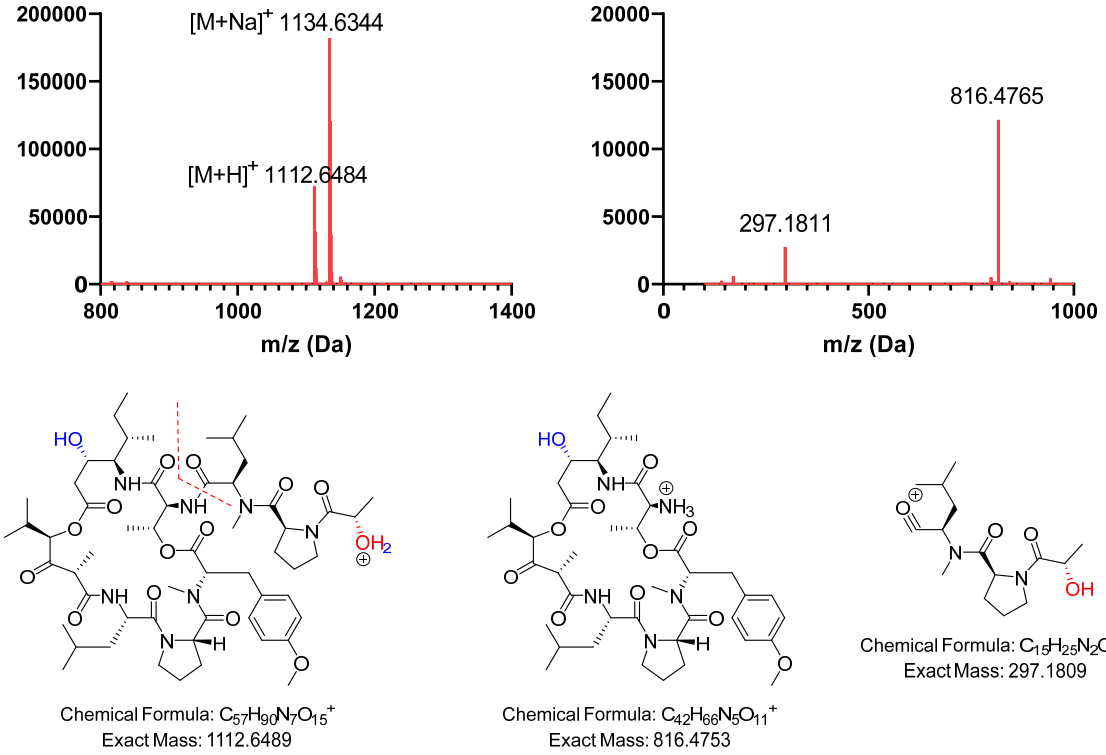
Supplementary Fig. 29 ^{1}H-^{1}H COSY spectrum of compound **6**. Solvent: $CDCl_3$.



Supplementary Fig. 30 ^{1}H-^{13}C HSQC spectrum of compound **6**. Solvent: $CDCl_3$.



Supplementary Fig. 31 HRMS and MS^2 analysis of didemnin B (**2**).

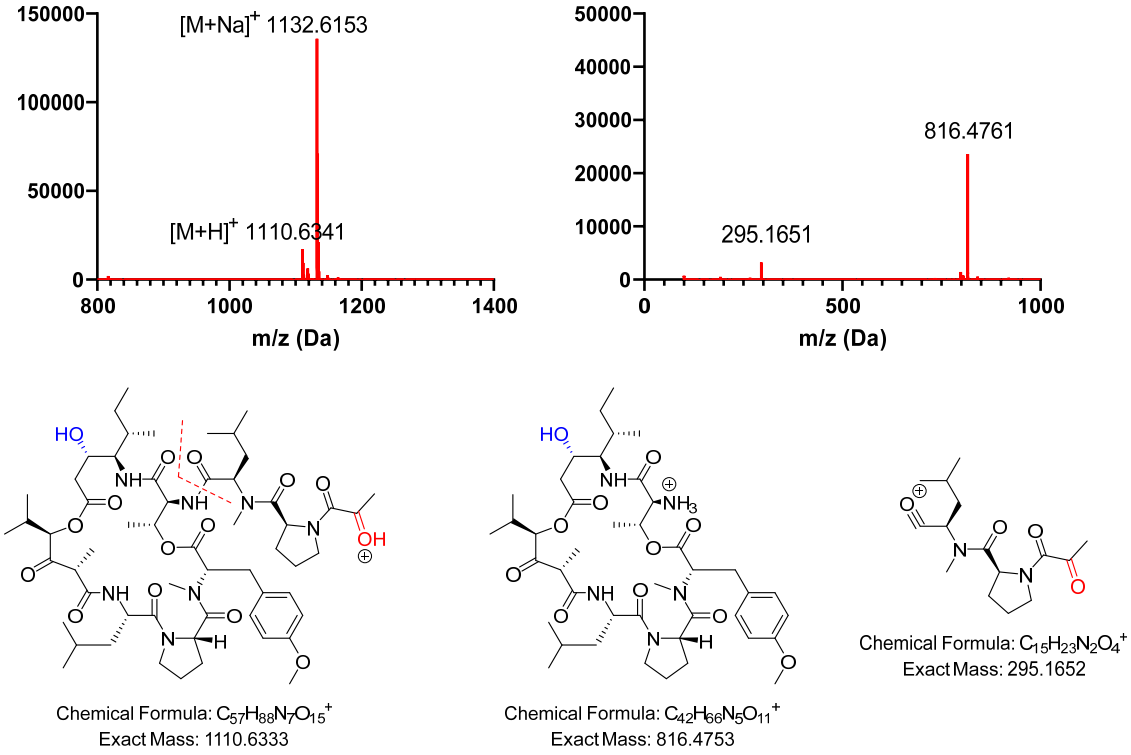


Chemical Formula: $C_{57}H_{90}N_7O_{15}^+$
Exact Mass: 1112.6489

Chemical Formula: $C_{42}H_{66}N_5O_{11}^+$
Exact Mass: 816.4753

Chemical Formula: $C_{15}H_{25}N_2O_4^+$
Exact Mass: 297.1809

Supplementary Fig. 32 MS and MS^2 analysis of plitidepsin (**1**).

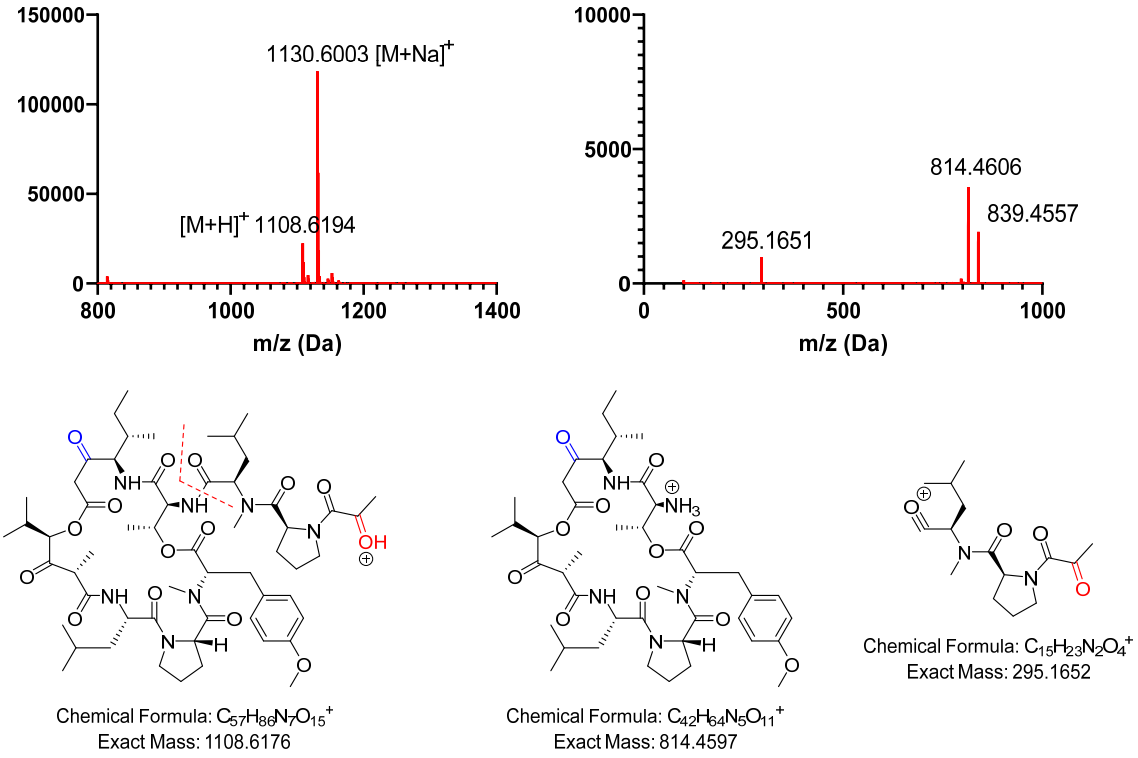


Chemical Formula: $C_{57}H_{88}N_7O_{15}^+$
Exact Mass: 1110.6333

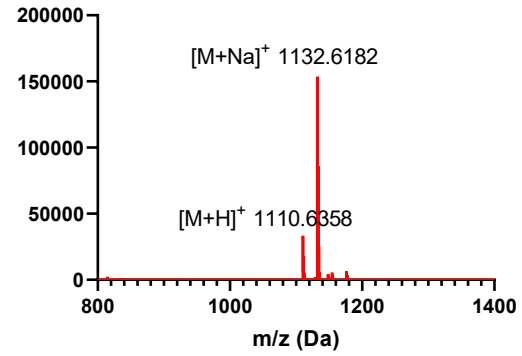
Chemical Formula: $C_{42}H_{66}N_5O_{11}^+$
Exact Mass: 816.4753

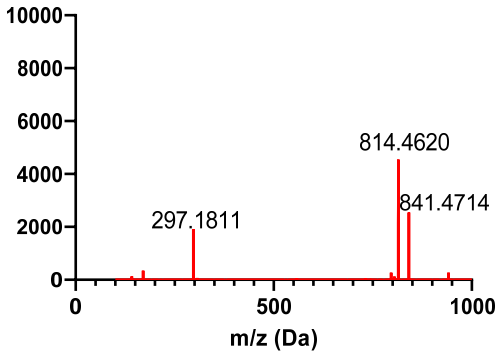
Chemical Formula: $C_{15}H_{23}N_2O_4^+$
Exact Mass: 295.1652

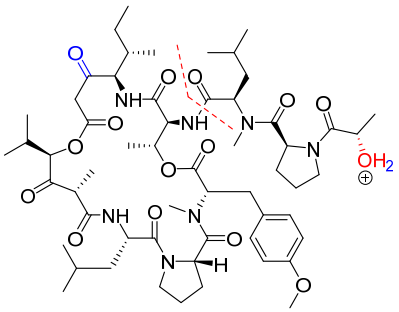
Supplementary Fig. 33 MS and MS^2 analysis of compound **5**.



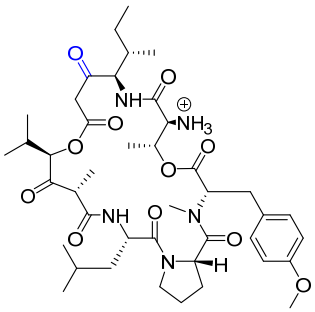
Supplementary Fig. 34 MS and MS^2 analysis of compound **6**.



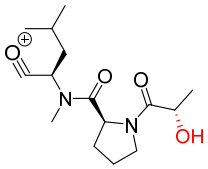




Chemical Formula: $C_{57}H_{88}N_7O_{15}^+$
Exact Mass: 1110.6333

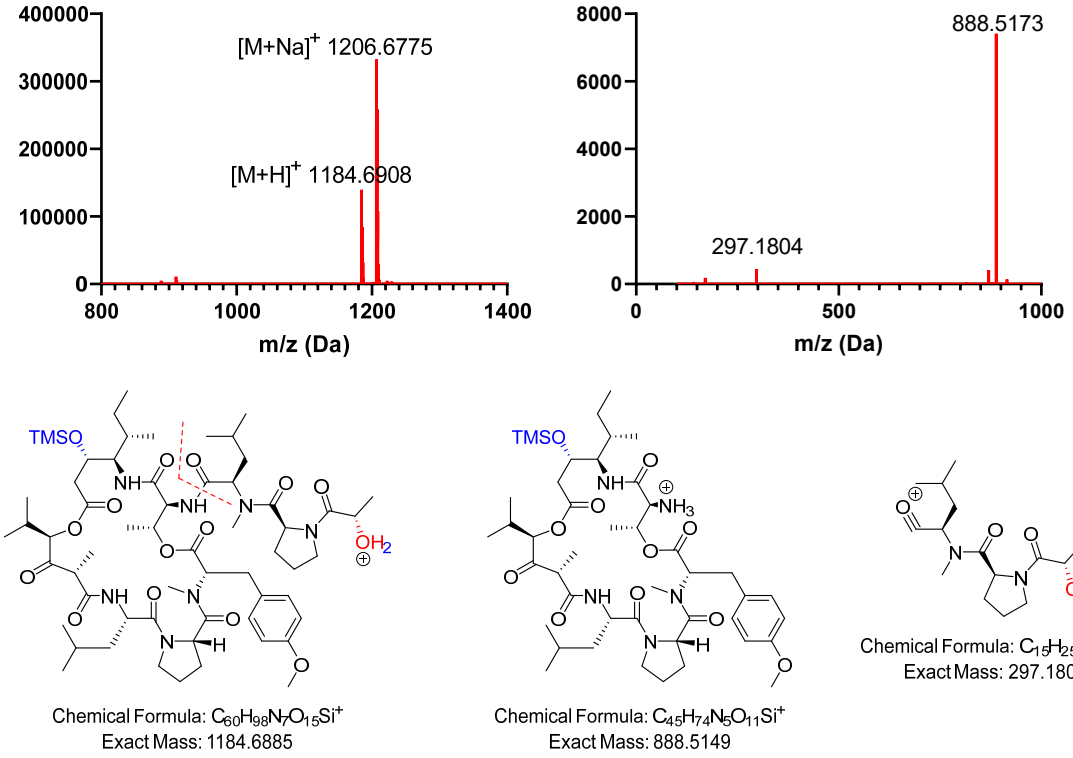


Chemical Formula: $C_{42}H_{64}N_5O_{11}^+$
Exact Mass: 814.4597



Chemical Formula: $C_{15}H_{25}N_2O_4^+$
Exact Mass: 297.1809

Supplementary Fig. 35 MS and MS^2 analysis of compound **3**.

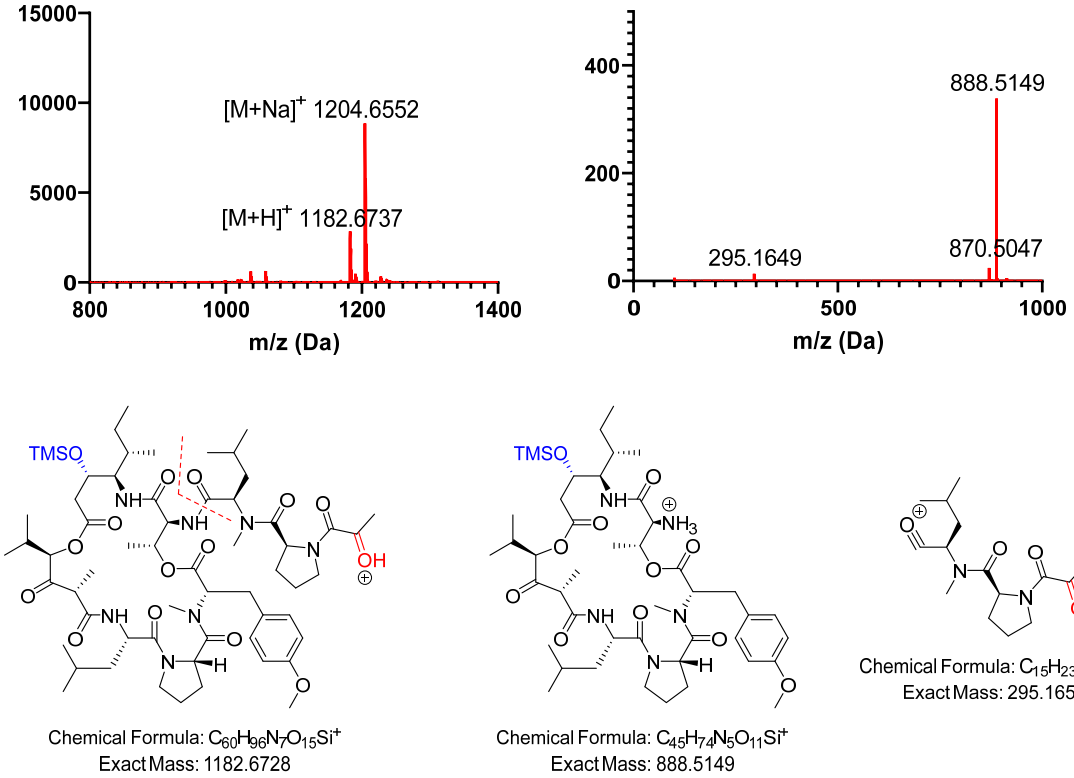


Chemical Formula: $C_{60}H_{98}N_7O_{15}Si^+$
Exact Mass: 1184.6885

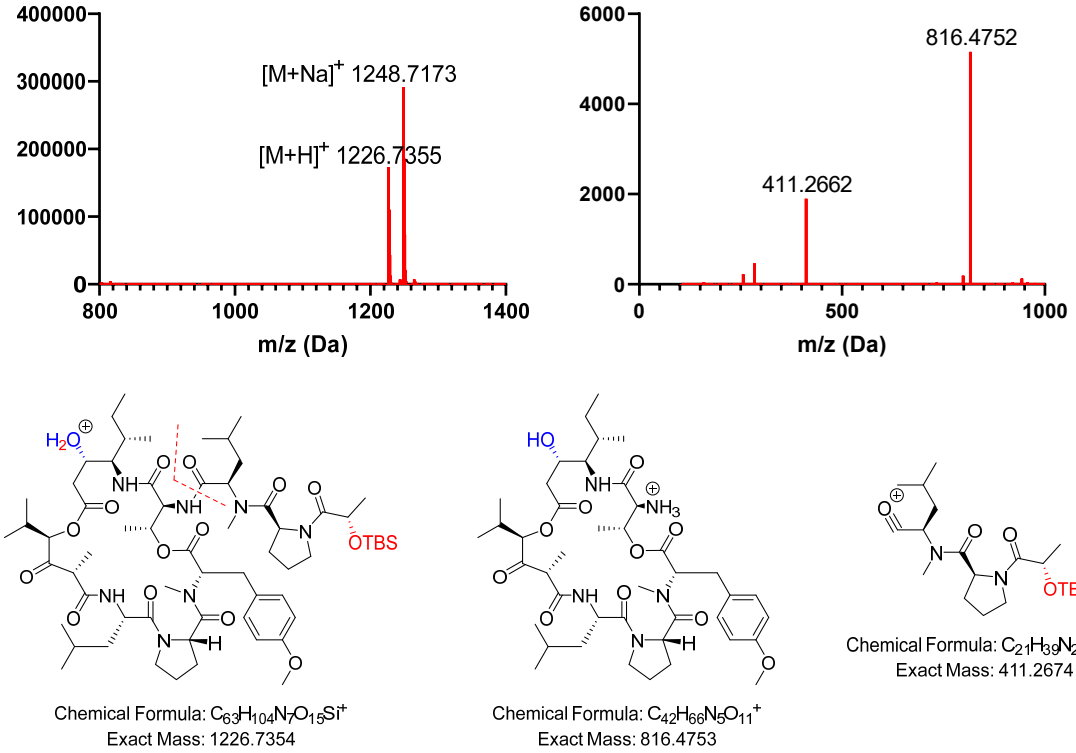
Chemical Formula: $C_{45}H_{74}N_5O_{11}Si^+$
Exact Mass: 888.5149

Chemical Formula: $C_{15}H_{25}N_2O_4^+$
Exact Mass: 297.1809

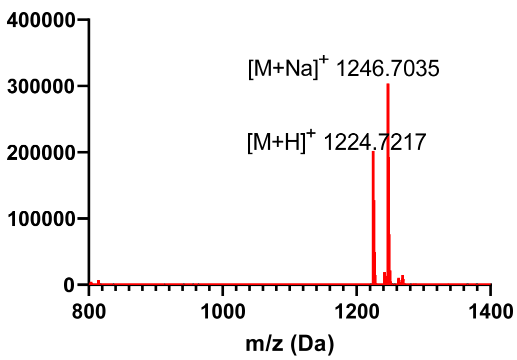
Supplementary Fig. 36 MS and MS^2 analysis of compound **4**.

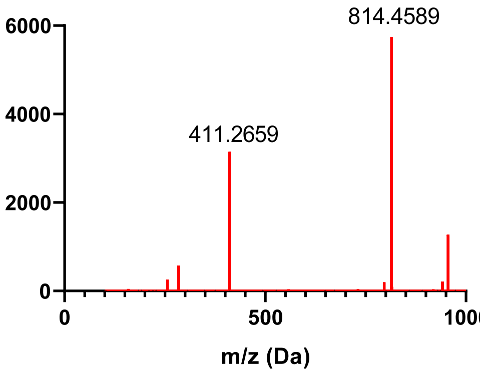


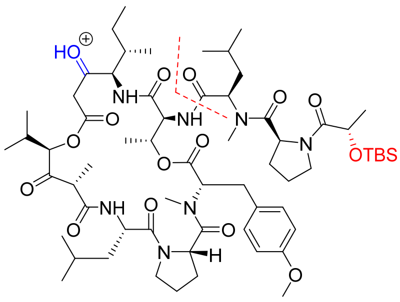
Supplementary Fig. 37 MS and MS^2 analysis of compound **7**.



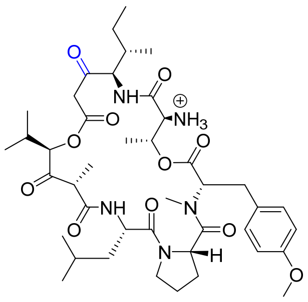
Supplementary Fig. 38 MS and MS^2 analysis of compound **8**.



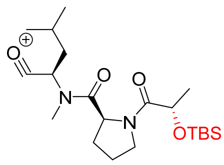




Chemical Formula: $C_{63}H_{102}N_7O_{15}Si^+$
Exact Mass: 1224.7198

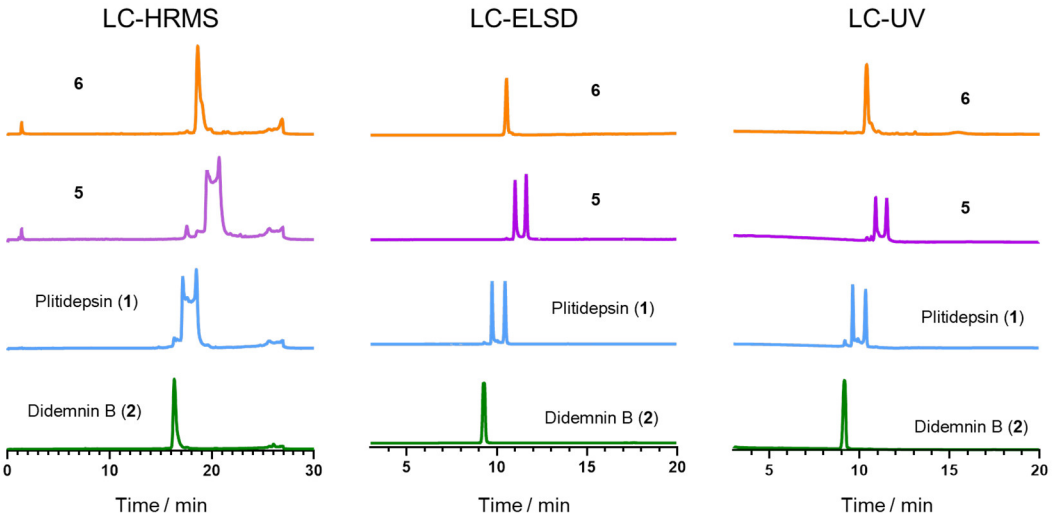


Chemical Formula: $C_{42}H_{64}N_5O_{11}^+$
Exact Mass: 814.4597



Chemical Formula: $C_{21}H_{39}N_2O_4Si^+$
Exact Mass: 411.2674

Supplementary Fig. 39 LC-HRMS chromatography, LC-ELSD chromatography and UV spectra (210 nm) of the purified compounds **1**, **2**, **5**, and **6**.



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[2] K. Yamanaka, K. A. Reynolds, R. D. Kersten, K. S. Ryan, D. J. Gonzalez, V. Nizet, P. C. Dorrestein, B. S. Moore, *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, 1957–1962.

[3] R. Sakai, K. L. Rinehart, V. Kishore, B. Kundu, G. Faircloth, J. B. Gloer, J. R. Carney, M. Namikoshi, F. Sun, R. G. Hughes, D. G. Grávalos, T. G. De Quesada, G. R. Wilson, R. M. Heid, *J. Med. Chem.* **1996**, *39*, 2819–2834.

[4] M. Tsukimoto, M. Nagaoka, Y. Shishido, J. Fujimoto, F. Nishisaka, S. Matsumoto, E. Harunari, C. Imada, T. Matsuzaki, *J. Nat. Prod.* **2011**, *74*, 2329–2331.