Supporting Information

Asymmetric synthesis of Fmoc-threo-HOAsn for the total synthesis of Nicrophorusamides A

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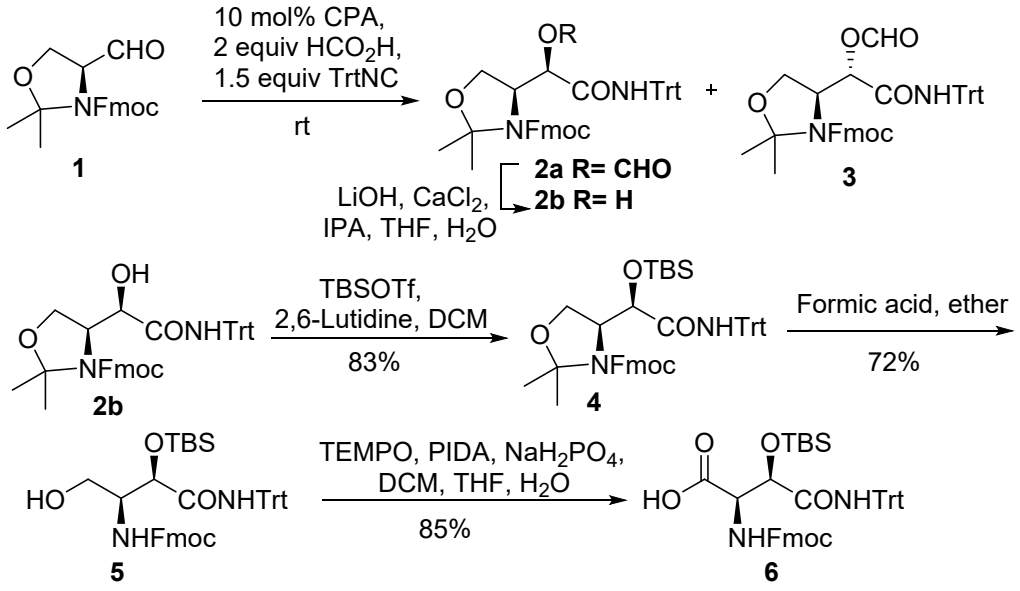
1、General Method

All commercially available materials were used without further purification. Amino acids, coupling reagents and resins were obtained from GL Biochem, CS Bio and Chem-Impex unless otherwise specified. All solvents were reagent grade or HPLC grade. Anhydrous solvents were either prepared from AR grade solvents via standard methods (DCM), or purchased in anhydrous form (DMF). Analytical reversed-phase high-performance liquid chromatography (RP-HPLC) separations involving a mobile phase of acetonitrile (Solvent A) and 0.1% TFA (v/v) in water (Solvent B) were performed on an Agilent UPLC system equipped with a 1260 Infinity detector and an Agilent 6120 Quadrupole LC/MS system using an Agilent Poroshell 120 EC-C18 (2.7 μm, 3.0 x 50 mm). Preparative HPLC separations involving a mobile phase of acetonitrile (Solvent A) and 0.1% TFA (v/v) in water (Solvent B) were performed on a Shimadzu HPLC system equipped with a binary pump (LC-20AR) and a UV/Vis detector (SPD-20A) using a YMC-Pack ODS-A (5 μm, 120 Å , 250 x 20 mml) at a flow rate of 8 mL/min. Analytical TLC was performed on HSGF254 silica gel plates (0.2mm) purchased from Yantai Jiangyou Silicone Development Co., Ltd (China) and visualized under UV light (254 nm) or by staining with ninhydrin. The flash column chromatography was performed using silica gel (200–300 meshes) purchased from the Anhui Liangchen Silicon Material Company (China). The ^{1}H and ^{13}C NMR spectra were recorded on JEOL 400 and 600 MHz spectrometers and Bruker 600 MHz spectrometers. The high-resolution mass spectrometry (HRMS) was performed on a Waters Micromass Q-TOF Premier Mass Spectrometer.

2、Experimental Procedures on Building Blocks Syntheses

2.1. Synthesis of HOAsn building block



*The general procedure for synthesis of (9H-fluoren-9-yl)methyl-(S)-4-((R)-1-(formyloxy)-2-oxo-2-(tritylamino)ethyl)-2,2-dimethyloxazolidine-3-carboxylate (**2a**)*. To a solution of aldehyde **1** (100 mg, 0.28 mmol, 1.0 equiv) in toluene (3.0 mL), TrtNC (115

mg, 0.42 mmol, 1.5 equiv) and formic acid (21.50 μL, 0.56 mmol, 2 equiv) were added sequentially and the reaction mixture was stirred at 60 °C for 30 min. The reaction mixture was concentrated under vacuo and purified by flash column chromatography on silica gel (hexane/EtOAc, 3:1) to give compound **2a** (85.4 mg, 45%), compound **2b** (63.6 mg, 35%) and compound **3** (3.80 mg, 2%)as white solid. Compound **2a:** ^{1}H NMR (600 MHz, Acetone-d_6) δ 8.10 (s, 1H), 7.90 - 7.76 (m, 4H), 7.69 (s, 2H), 7.42 - 7.34 (m, 3H), 7.32 - 7.19 (m, 23H), 5.38 (s, 1H), 4.68 (s, 1H), 4.47 (s, 1H), 4.28 (d, J = 4.8 Hz, 1H), 4.21 (t, J = 6.6 Hz, 1H), 3.90 (s, 1H), 1.51 - 1.34 (m, 3H), 0.98 - 0.72 (m, 3H). ^{13}C NMR (125 MHz, Acetone-d_6) δ 166.74, 160.84, 145.47, 145.31, 142.22, 129.72, 128.50, 128.47, 128.00, 127.69, 120.79, 95.55, 73.05, 71.37, 65.05, 60.54, 48.07, 29.84, 26.42, 20.82, 14.48. HRMS (ESI$^+$) calcd. for $C_{42}H_{38}N_2O_6$ [M+Na]$^+$ *m/z*: 689.2622; found: 689.2625. Compound **3**: ^{1}H NMR (600 MHz, Chloroform-d) δ 8.07 (s, 2H), 7.77 - 7.68 (m, 4H), 7.62 - 7.53 (m, 4H), 7.41 – 7.36 (m, 4H), 7.35 - 7.27 (m, 20H), 7.23 (dd, J = 13.3, 7.1 Hz, 4H), 7.20 - 7.15 (m, 13H), 5.73 (s, 1H), 5.58 (s, 1H), 4.80 - 4.71 (m, 2H), 4.59 (dd, J = 10.4, 6.2 Hz, 1H), 4.47 (dd, J = 10.3, 6.5 Hz, 1H), 4.35 (s, 1H), 4.24 – 4.19 (m, 3H), 3.92 – 3.85 (m, 3H), 3.69 (t, J = 8.1 Hz, 1H), 1.53 (s, 3H), 1.44 (s, 3H), 0.85 (s, 3H), 0.78 (s, 3H). ^{13}C NMR (150 MHz, Chloroform-d) δ 165.84, 165.63, 160.12, 159.68, 153.10, 152.29, 144.14, 144.09, 143.88, 143.76, 141.73, 141.67, 141.48, 141.36, 128.64, 128.26, 128.17, 127.86, 127.49, 127.33, 125.13, 125.04, 124.50, 124.33, 120.16, 95.32, 94.29, 72.48, 72.11, 70.77, 67.15, 66.45, 64.26, 63.21, 58.42, 57.63, 47.40, 29.84, 29.46, 25.89, 25.62, 23.55, 23.11. HRMS (ESI$^+$) calcd. for $C_{42}H_{38}N_2O_6$ [M+Na]$^+$ m/z: 689.2622; found: 689.2625.

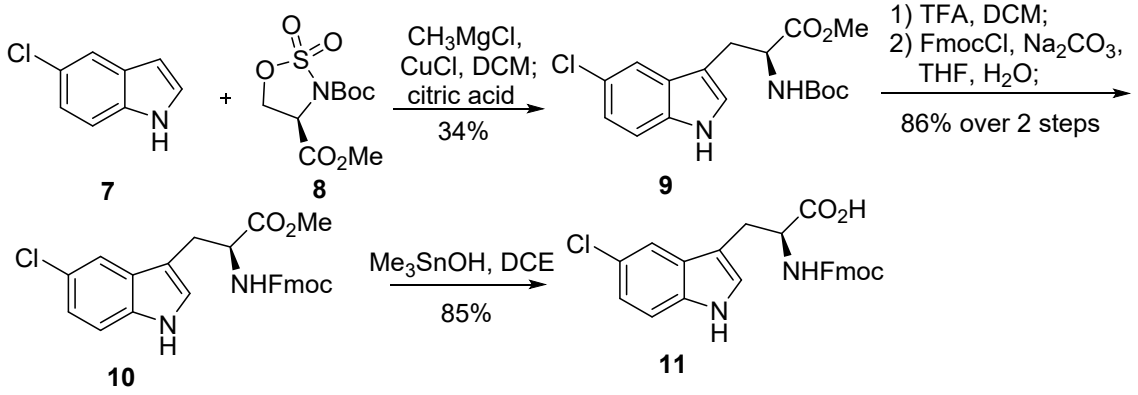
*The procedure for synthesis of (9H-fluoren-9-yl)methyl-(S)-4-((R)-1-hydroxy-2-oxo-2-(tritylamino)ethyl)-2,2-dimethyloxazolidine-3-carboxylate (**2b**).* To a stirred solution of compound **2a** (5.93 g, 8.90 mmol, 1.0 equiv.) in isopropanol (42 mL) and THF (14 mL), $CaCl_2$ (14.81 g, 133.44 mmol, 15.0 equiv) was added. After the $CaCl_2$ was dissolved, $LiOH{\cdot}H_2O$ (1.86 g, 44.50 mmol, 5.0 equiv.) in H_2O (14mL) was added dropwise. The reaction mixture was stirred at rt for 1.5 h. The mixture was acidified by 1 N HCl to neutral at 0 °C. The aqueous layer was extracted with EtOAc (150 mL x 2). The combined organic layer was washed with brine (100 mL x 1). The organic phase was dried over Na_2SO_4, concentrated under vacuo and purified by flash column chromatography on silica gel (hexane/EtOAc, 3:1) to give compound **2b** (5.08 g, 89%) as white solid. Compound **2b:** ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.22 (s, 1H), 8.06 (s, 1H), 7.87 (d, J = 7.2 Hz, 4H), 7.65 (s, 4H), 7.37 (t, J = 7.2 Hz, 4H), 7.32 - 7.16 (m, 26H), 7.13 (s, 8H), 6.48 (s, 1H), 6.34 (s, 1H), 4.75 (d, J = 9.0 Hz, 1H), 4.59 (d, J = 8.4 Hz, 1H), 4.36 (s, 1H), 4.28 (s, 2H), 4.22 - 3.73 (m, 9H), 1.50 (s, 2H), 1.41 (s, 3H), 0.71 (s, 3H), 0.59 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.32, 169.99, 152.44, 151.97, 144.66, 144.16, 141.10, 141.00, 140.70, 140.58, 128.36, 127.72, 127.11, 127.09, 126.72, 124.40, 119.99, 94.20, 93.76, 69.84, 69.14, 69.06, 66.60, 65.80, 64.22, 63.20, 60.14, 59.33, 46.59, 30.95, 24.69, 22.78, 22.06, 13.96. HRMS (ESI$^+$) calcd. for $C_{41}H_{38}N_2O_5$ [M+Na]$^+$ *m/z*: 661.2673; found: 661.2680.

The procedure for synthesis of (9H-fluoren-9-yl)methyl-(S)-4-((R)-1-((tert-butyldimethylsilyl)oxy)-2-oxo-2-(tritylamino)ethyl)-2,2-dimethyloxazolidine-3-

carboxylate (4). To a 100 mL dry round bottom flask, compound **2b** (5.00 g, 7.83mmol, 1.0 equiv.) was added. After argon protection of the flask, anhydrous DCM (50mL) was added, and the mixture was stirred until compound **2b** dissolved. After the stirred solution cooled to 0 °C, 2,6-lutidine (4.56 mL, 39.16 mmol, 5.0 equiv.) was added and followed by TBSOTf (4.50 mL, 19.58 mmol, 2.5 equiv.) dropwise. The reaction mixture was stirred at 0 °C for 1 h, then diluted with DCM (150 mL), washed with aqueous HCl (100 mL, 1 M), saturated $NaHCO_3$ (100 mL), brine (100 mL). The organic phase was dried over Na_2SO_4, concentrated under vacuo. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc, 8:1) to give compound **4** (4.89 g, 83%) as white solid. Compound **4:** ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.89 (s, 1H), 7.85 (s, 1H), 7.74 (d, *J* = 7.2 Hz, 3H), 7.66 (d, *J* = 7.8 Hz, 1H), 7.54 (dd, *J* = 13.2, 7.8 Hz, 3H), 7.39 - 7.33 (m, 3H), 7.32 - 7.26 (m, 10H), 7.25 - 7.20 (m, 15H), 4.73 (dd, *J* = 10.8, 4.8 Hz, 1H), 4.57 (dd, *J* = 10.8, 4.2 Hz, 1H), 4.53 - 4.44 (m, 2H), 4.41 - 4.34 (m, 1H), 4.21 - 4.11 (m, 3H), 4.08 (dd, *J* = 9.6, 6.6 Hz, 1H), 4.01 (t, *J* = 5.4 Hz, 1H), 3.94 (t, *J* = 6.0 Hz, 1H), 3.89 - 3.85 (m, 1H), 1.67 (s, 2H), 1.53 (s, 2H), 0.99 (s, 3H), 0.81 (s, 3H), 0.79 (s, 10H), 0.77 (s, 5H), 0.13 (s, 3H), 0.09 (s, 3H), -0.12 (s, 2H), -0.14 (s, 2H). ^{13}C NMR (150 MHz, Chloroform-*d*) δ 169.10, 153.46, 144.77, 144.50, 144.14, 144.04, 143.76, 141.76, 141.49, 128.92, 128.01, 127.92, 127.75, 127.39, 127.22, 127.13, 127.05, 125.91, 125.16, 124.66, 124.55, 120.03, 119.96, 96.09, 95.20, 71.60, 71.08, 70.37, 70.30, 67.72, 66.56, 63.74, 63.12, 61.57, 60.70, 47.29, 29.83, 25.64, 25.60, 25.33, 23.27, 22.95, 17.72, -4.80, -4.93, -4.98. HRMS (ESI$^+$) calcd. for $C_{47}H_{52}N_2O_5Si$ $[M+Na]^+$ *m/z*: 775.3538; found: 775.3539.

*The procedure for synthesis of (9H-fluoren-9-yl)methyl-((2S,3R)-3-((tert-butyldimethylsilyl)oxy)-1-hydroxy-4-oxo-4-(tritylamino)butan-2-yl)carbamate (**5**).* To a stirred solution of compound **4** (4.50 g, 5.98 mmol, 1.0 equiv.) in formic acid / ether (50 mL, 2:3). The mixture was stirred at rt for 4 h. The reaction mixture was concentrated under vacuo. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc, 2:1) to give compound **5** (3.07 g, 72 %) as white solid. Compound **5:** ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.03 (s, 1H), 7.76 (d, *J* = 7.2 Hz, 2H), 7.58 (t, *J* = 7.8 Hz, 2H), 7.39 (t, *J* = 7.8 Hz, 2H), 7.30 (m, 13H), 7.22 (d, *J* = 7.2 Hz, 7H), 5.94 (d, *J* = 9.6 Hz, 1H), 4.44 - 4.39 (m, 1H), 4.35 - 4.30 (m, 1H), 4.27 (d, *J* = 4.2 Hz, 1H), 4.17 (t, *J* = 7.2 Hz, 1H), 4.06 - 4.00 (m, 1H), 3.71 - 3.66 (m, 1H), 3.63 - 3.57 (m, 1H), 0.90 (s, 10H), 0.20 (s, 3H), 0.12 (s, 3H). ^{13}C NMR (150 MHz, Chloroform-*d*) δ 171.17, 156.69, 144.24, 143.97, 141.38, 128.73, 128.19, 127.76, 127.43, 127.17, 127.14, 125.31, 125.21, 120.04, 120.03, 72.72, 70.63, 67.24, 62.25, 56.67, 47.27, 25.76, 17.91, -4.89. HRMS (ESI$^+$) calcd. for $C_{44}H_{48}N_2O_5Si$ $[M+Na]^+$ *m/z*: 735.3225; found: 735.3221.

2.2. Synthesis of 5-Cl-Trp building block



*The procedure for synthesis of (2R,3R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-((tert-butyldimethylsilyl)oxy)-4-oxo-4-(tritylamino)butanoic acid (**6**).* To a stirred solution of compound **5** (3.00 g, 4.21 mmol, 1.0 equiv.) in DCM/THF/H_2O (40 mL, 2:2:1), NaH_2PO_4 (2.53 g, 21.06 mmol, 5.0 equiv.), TEMPO (131 mg, 0.84 mmol, 0.2 equiv.) and PIDA (6.78 g, 21.06 mmol, 5.0 equiv.) was added sequentially. The reaction mixture was stirred at rt for 1 h. The reaction mixture was diluted with EtOAc (150 mL), washed with sat. $Na_2S_2O_3$ (50 mL x 2), brine (50 mL x 1). The organic phase was dried over Na_2SO_4, concentrated under vacuo and purified by flash column chromatography on silica gel (hexane/EtOAc, 2:1 with 1% AcOH) to give compound **6** (2.60 g, 85%) as colorless solid. Compound **6:** ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.17 (s, 1H), 7.89 (t, J = 6.6 Hz, 2H), 7.79 (d, J = 7.2 Hz, 1H), 7.74 (d, J = 7.8 Hz, 1H), 7.44 - 7.36 (m, 2H), 7.29 (t, J = 7.2 Hz, 1H), 7.26 - 7.14 (m, 18H), 4.61 (d, J = 1.8 Hz, 1H), 4.53 - 4.45 (m, 1H), 4.38 (dd, J = 9.0, 1.2 Hz, 1H), 4.26 (t, J = 7.2 Hz, 1H), 4.23 - 4.17 (m, 1H), 0.88 (d, J = 16.8 Hz, 9H), 0.08 - 0.00 (m, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.05, 169.78, 156.23, 156.11, 144.75, 144.37, 143.88, 143.82, 140.67, 140.62, 128.41, 127.66, 127.60, 127.09, 126.72, 126.66, 125.34, 120.06, 74.61, 72.81, 69.43, 69.08, 66.14, 65.79, 58.11, 57.88, 46.70, 39.52, 25.82, 25.71, 17.81, 17.66, -3.18, -4.88, -5.16. HRMS (ESI$^+$) calcd. for $C_{44}H_{46}N_2O_6Si$ $[M+Na]^+$ *m/z*: 749.3017; found: 749.3017.

*The procedure for synthesis of tert-butyl (R)-3-(2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)-5-chloro-1H-indole-1-carboxylate (**9**).*[12] Compound **7** (2.28 g, 15.10 mmol, 1.5 equiv) and CuCl (1.30 g, 13.08 mmol, 1.3 equiv) were added to a dry round bottom flask and argon protection was completed. Dry DCM (50mL) was added and stirred at 0 °C over 10 min, MeMgCl (3 M in THF, 4.36 mL, 13.09 mmol, 1.3 equiv) was added dropwise, the reaction mixture was stirred at 0 °C for 1 h and then cooled to -20 °C. A solution of sulfamidates **8** (2.83 g, 10.07 mmol, 1.0 equiv) in dry DCM (10 mL) was added dropwise to the reaction mixture at -20 °C for 30 min. The reaction was allowed to return to room temperature and stirred for 18 h. Then 2 M citric acid solution (100 mL) was added dropwise at 0 °C and stirred at room temperature for 3 h. The reaction mixture was extracted with DCM (150 mL x 2), then washed with brine (100 mL x 1), dried over Na_2SO_4, concentrated under vacuo and purified by flash column

chromatography on silica gel (hexane/EtOAc, 2:1) to give compound **9** (1.21 g, 34%) as white solid. Compound **9:** ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.29 (s, 1H), 7.49 (d, *J* =1.2 Hz,1H), 7.24 (d, *J* = 8.4 Hz, 1H), 7.14 - 7.10 (m, 1H), 7.00 (s, 1H), 5.10 (d, *J* = 7.2 Hz, 1H), 4.67 - 4.60 (m, 1H), 3.70 (s, 3H), 3.29 - 3.19 (m, *J* = 14.9, 5.4 Hz, 2H), 1.44 (s, 9H). ^{13}C NMR (150 MHz, Chloroform-*d*) δ 172.64, 155.29, 134.60, 128.95, 125.54, 124.35, 122.60, 118.48, 112.35, 110.21, 80.18, 77.16, 54.33, 52.45, 28.45, 28.12. HRMS (ESI^{+}) calcd. for $C_{17}H_{21}ClN_2O_4$ $[M+Na]^{+}$ *m/z*: 375.1082; found: 375.1083.

*The procedure for synthesis of tert-butyl (R)-3-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methoxy-3-oxopropyl)-5-chloro-1H-indole-1-carboxylate (**10**).* To a stirred solution of compound **9** (1.21 g, 3.44 mmol, 1.0 equiv.) in TFA/DCM (18 mL, 1:5). The mixture was stirred at rt for 30 min. The reaction mixture was concentrated under vacuo to remove TFA and DCM, no purification required. The reaction mixture was further dissolved in THF/H_2O (24 mL, 1:2), Na_2CO_3 (1.09 g, 10.28 mmol, 3.0 equiv.) and Fmoc-Cl (1.16 g, 4.48 mmol, 1.3 equiv.) was added slowly and stirred at rt for 1.5 h. The reaction mixture was extracted with DCM (100 mL), washed with sat. NH_4Cl (50 mL x 2), brine (50 mL x 1), dried over Na_2SO_4, concentrated under vacuo and purified by flash column chromatography on silica gel (hexane/EtOAc, 2:1) to give compound **10** (1.40 g, 86 % for two steps) as white solid. compound **10:** ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.20 (s, 1H), 7.76 (d, *J* = 7.2 Hz, 2H), 7.58 - 7.51 (m, 3H), 7.40 (t, *J* = 7.2 Hz, 2H), 7.30 (q, *J* = 6.6 Hz, 2H), 7.24 (d, *J* = 8.4 Hz, 1H), 7.13 (dd, *J* = 8.4, 1.2 Hz, 1H), 6.93 (s, 1H), 5.38 (d, *J* = 8.4 Hz, 1H), 4.74 - 4.68 (m, 1H), 4.46 (dd, *J* = 10.2, 6.6 Hz, 1H), 4.37 (dd, *J* = 10.2, 7.2 Hz, 1H), 4.21 (t, *J* = 6.6 Hz, 1H), 3.72 (s, 3H), 3.34 - 3.23 (m, 2H). ^{13}C NMR (150 MHz, Chloroform-*d*) δ 172.33, 155.86, 143.96, 141.44, 134.55, 128.84, 127.85, 127.23, 125.65, 125.26, 125.18, 124.44, 122.70, 120.11, 118.25, 112.44, 109.84, 77.16, 67.13, 54.52, 52.61, 47.29, 27.88. HRMS (ESI^{+}) calcd. for $C_{27}H_{23}ClN_2O_4$ $[M+Na]^{+}$ *m/z*: 497.1239; found: 497.1241.

*The procedure for synthesis of (R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(5-chloro-1H-indol-3-yl)propanoic acid (**11**).* To a stirred solution of compound **10** (1.40 g, 2.95 mmol, 1.0 equiv.) and Me_3SnOH (3.20 g, 17.70 mmol, 6.0 equiv.) in 1,2-DCE (30 mL). The mixture was stirred at 70 °C for 4 h. The reaction mixture was quenched with aqueous $KHSO_4$ (0.01N) and was extracted with DCM (100 x 2 mL). The organic layer was washed with brine (50 x 2 mL), dried over Na_2SO_4, concentrated under vacuo and purified by flash column chromatography on silica gel (hexane/EtOAc, 2:1 with 1% AcOH) to give compound **11** (1.16 g, 85%) as white solid. Compound **11:** ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.00 (s, 1H), 7.87 (d, *J* = 7.8 Hz, 2H), 7.66 (t, *J* = 7.2 Hz, 2H), 7.55 (s, 1H), 7.42 - 7.38 (m, 2H), 7.33 (d, *J* = 8.4 Hz, 1H), 7.32 - 7.26 (m, 2H), 7.14 (s, 1H), 7.09 (s, 1H), 7.04 (dd, *J* = 8.4, 1.8 Hz, 1H), 4.19 - 4.16 (m, 3H), 4.09 - 4.04 (m, 1H), 3.17 (d, *J* = 5.4 Hz, 1H), 3.11 (dd, *J* = 14.4, 4.2 Hz, 1H), 2.97 (dd, *J* = 14.4, 8.4 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.16, 155.86, 143.80, 140.65, 139.43, 137.44, 134.53, 128.93, 128.61, 127.60, 127.29, 127.06, 125.55, 125.30, 125.21, 123.09, 121.38, 120.74, 120.08, 120.03, 117.61, 112.88, 110.65, 66.36, 65.60, 54.91, 46.63, 39.52, 26.86. HRMS (ESI^{+}) calcd. for $C_{26}H_{21}ClN_2O_4$ $[M+Na]^{+}$ *m/z*: 483.1082; found: 483.1083.

3. General Procedures of Fmoc-based solid-phase peptide synthesis (SPPS) of Nicrophorusamides A and analogues

The solid phase peptide synthesis was carried out manually using 2-chlorotrityl chloride resin (loading: ~0.78 mmol/g) under the standard Fmoc-SPPS protocols starting with L-Orn, added L-*allo*-Ile, D-Leu, D-Val, 5-Cl-L-Trp in order and finally D-HOAsn. Thereafter, we replace 5-Cl-L-Trp with L-Trp, L-Pro and L-Phe, then using the D-Thr to replace D-HOAsn. The crude peptide with side chain protected groups attached was cleaved from the resin after final Fmoc deprotection. The Fmoc deprotection was carried out using 20% piperidine in DMF.

Loading of Fmoc-L-Orn(Boc)-OH to 2-chlorotrityl chloride resin

The 2-chlorotrityl chloride resin (100 mg, loading: 0.78 mmol/g) was swollen in dry DCM for 20 min in a 10 mL disposable vessel (HENKE-JECT) equipped with a porous polypropylene disc at the bottom. A solution of Fmoc-L-Orn(Boc)-OH (4.0 equiv.) and DIEA (4.0 equiv.) in DCM (6 mL) was added and the reaction vessel was shaken on the vortex at room temperature for 1 h. The resin was washed with DMF (5 × 6 mL) followed by a solution of DCM/MeOH/DIEA (17:2:1, v/v/v, 6 mL) for 20 min and washed with DMF (5 × 6 mL). The resin was subsequently submitted to iterative peptide assembly via the standard Fmoc-SPPS protocol.

Fmoc deprotection

The Fmoc deprotection was carried out using 6 mL of 20% piperidine in DMF at room temperature for 20 min. The resin was then washed with DMF (5 × 6 mL).

Procedure of coupling of amino acids

For the coupling step other than the two unnatural amino acid building blocks, a solution of Fmoc amino acid (4 equiv.), HATU (4 equiv.), DIEA (8 equiv.) in DMF was gently agitated on vortex with the resin at room temperature for 1 h. The resin was then washed alternately with DCM (3 × 6 mL) and DMF (3 × 6 mL), followed by Fmoc-deprotection.

Procedure of coupling of Fmoc-HOAsn(OTBS,Trt)-OH and Fmoc-5-Cl-Trp-OH

A solution of Fmoc-amino acid building blocks (3 equiv.), HATU (3 equiv.), DIEA (6 equiv.) in DMF was gently agitated on vortex with the resin at room temperature for 5 h. The resin was then washed alternately with DCM (3 × 6 mL) and DMF (3 × 6 mL), followed by Fmoc-deprotection.

Cleavage of the crude protected peptide from the resin

The resin was washed with DMF (3 x 6 mL) and DCM (3 x 6 mL) and was subjected to 6 mL of mild acidic cleavage cocktail of DCM/AcOH/trifluoroethanol (8/1/1, v/v/v) for 1 h. Following filtration, the resulting cleavage solutions were combined and concentrated in vacuo to give the crude linear side chain protected peptide. The crude peptide was further dried by vacuum overnight prior to the macrocyclization.

Macrocyclization

To a solution of the crude peptide (1 equiv.) in dry DCM (1 mM) was added DIC (5 equiv.), HOAt (5 equiv.) and DIEA (10 equiv.). The solution was stirred at room temperature for 20 h. The solvent was removed in vacuo afterwards.

Global deprotection

A solution of 4N HCl (3 mL) was added and the solution was stirred at room temperature for 1 h. The solution was concentrated in vacuo. Followed by a solution of TFA (3 mL) was added and the solution was stirred at room temperature for 1 h. The solution was concentrated in vacuo and the crude product was precipitated by cold diethyl ether. Diethyl ether was discarded after centrifugation and the crude product was washed with cold diethyl ether for 3 times. The washed crude product was dried under vacuum to remove residual diethyl ether and the yellowish solid was ready for HPLC purification. The average yield after HPLC purification is 3 % based on resin loading.

Nicrophorusamides A **15**: 5.5 mg (from 300 mg resin), 3.0%. LC-MS (ESI^+): calculated for $C_{37}H_{56}ClN_9O_8$ $[M+H]^+$ *m/z:* 790.4; found: 790.5, $[M+2H]^{2+}$ *m/z:* 395.7; found: 395.8. (Figure S1). Compound **15**: ^1H NMR (400 MHz, DMSO-d_6) δ 11.06 (s, 1H), 8.46 (d, *J* = 4.8 Hz, 1H), 8.01 (d, *J* = 7.6 Hz, 1H), 7.86 (t, *J* = 10.4 Hz, 2H), 7.74 - 7.61 (m, 4H), 7.58 (s, 1H), 7.52 (d, *J* = 7.2 Hz, 1H), 7.36 - 7.28 (m, 3H), 7.15 (s, 1H), 7.04 (d, *J* = 8.8 Hz, 1H), 5.90 (d, *J* = 6.0 Hz, 1H), 4.59 (t, *J* = 5.2 Hz, 2H), 4.46 (d, *J* = 6.0 Hz, 1H), 4.28 - 4.18 (m, 2H), 4.01 (d, *J* = 6.8 Hz, 1H), 3.73 (t, *J* = 7.2 Hz, 1H), 3.10 (dd, *J* = 14.4, 7.2 Hz, 1H), 2.93 - 2.84 (m, 1H), 2.80 (s, 2H), 1.93 - 1.79 (m, 3H), 1.65 - 1.43 (m, 7H), 1.37 - 1.30 (m, 1H), 1.19 - 1.11 (m, 1H), 0.89 - 0.80 (m, 14H), 0.70 (dd, *J* = 12.4, 6.8 Hz, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.29, 172.08, 171.72, 171.09, 170.67, 170.41, 169.43, 134.48, 128.39, 125.47, 122.94, 120.70, 117.51, 112.77, 109.81, 70.85, 60.41, 56.45, 55.79, 53.57, 53.13, 51.60, 39.52, 38.43, 36.33, 29.14, 26.86, 26.79, 25.62, 24.36, 23.76, 22.82, 21.89, 18.89, 18.56, 14.51, 11.41.

Compound **16a**: 4.2 mg (from 100 mg resin), 7.1%. LC-MS (ESI^+): calculated for $C_{37}H_{57}N_9O_8$ $[M+H]^+$ *m/z:* 756.4 ; found: 756.5, $[M+2H]^{2+}$ *m/z:* 378.7; found: 378.9. (Figure S2). Compound **16a**: ^1H NMR (600 MHz, DMSO-d_6) δ 10.81 (s, 1H), 8.46 (d, *J* = 5.4 Hz, 1H), 8.00 (d, *J* = 7.8 Hz, 1H), 7.88 (d, *J* = 7.8 Hz, 1H), 7.77 (d, *J* = 7.2 Hz, 1H), 7.69 (s, 3H), 7.63 (d, *J* = 8.4 Hz, 1H), 7.53 (d, *J* = 7.8 Hz, 1H), 7.50 (d, *J* = 7.8 Hz, 1H), 7.33 – 7.29 (m, 3H), 7.09 (d, *J* = 1.8 Hz, 1H), 7.05 (t, *J* = 7.8 Hz, 1H), 6.97 (t, *J* = 7.2 Hz, 1H), 5.91 (d, *J* = 6.0 Hz, 1H), 4.65 – 4.59 (m, 2H), 4.47 – 4.44 (m, 1H), 4.26 – 4.21 (m, 2H), 4.02 (q, *J* = 7.2 Hz, 1H), 3.78 (t, *J* = 7.2 Hz, 1H), 3.51 (s, 1H), 3.17 (dd, *J* = 14.4, 6.6 Hz, 1H), 2.90 (dd, *J* = 14.4, 7.2 Hz, 1H), 2.82 – 2.76 (m, 2H), 2.07 (s, 1H), 1.92 – 1.83 (m, 3H), 1.69 – 1.61 (m, 1H), 1.60 – 1.55 (m, 2H), 1.55 – 1.45 (m, 3H), 1.35 (dt, *J* = 13.7, 6.8 Hz, 1H), 1.15 (dt, *J* = 14.4, 7.4 Hz, 1H), 0.85 (ddd, *J* = 15.1, 13.0, 6.4 Hz, 13H), 0.72 (dd, *J* = 15.0, 6.6 Hz, 6H).

Compound **16b**: 4.3 mg (from 100 mg resin), 8.3%. LC-MS (ESI^+): calculated for $C_{31}H_{54}N_8O_8$ $[M+H]^+$ *m/z:* 667.4; found: 667.5, $[M+2H]^{2+}$ *m/z:* 334.2; found: 334.3. (Figure S3). Compound **16b**: ^1H NMR (600 MHz, DMSO-d_6) δ 8.06 (d, *J* = 10.2 Hz,

1H), 7.94 (s, 1H), 7.89 (d, *J* = 6.0 Hz, 1H), 7.71 (d, *J* = 5.4 Hz, 1H), 7.60 (s, 5H), 7.31 (s, 1H), 7.22 (s, 1H), 5.64 (s, 1H), 4.78 (t, *J* = 7.8 Hz, 1H), 4.35 (dd, *J* = 7.8, 3.6 Hz, 1H), 4.23 (q, *J* = 7.2 Hz, 1H), 4.20 – 4.13 (m, 3H), 4.09 (dd, *J* = 8.4, 3.6 Hz, 1H), 4.07 – 4.03 (m, 1H), 4.00 – 3.95 (m, 1H), 3.63 – 3.58 (m, 2H), 2.82 – 2.76 (m, 3H), 2.62 – 2.60 (m, 1H), 2.39 – 2.37 (m, 1H), 2.12 – 2.06 (m, 1H), 1.96 – 1.85 (m, 5H), 1.81 – 1.74 (m, 4H), 1.58 – 1.47 (m, 8H), 1.25 – 1.08 (m, 5H), 1.00 (d, *J* = 6.6 Hz, 2H), 0.93 (d, *J* = 6.0 Hz, 4H), 0.85 (d, *J* = 6.6 Hz, 5H), 0.84 – 0.78 (m, 13H), 0.70 (d, *J* = 7.2 Hz, 4H).

Compound **16c**: 4.4 mg (from 100 mg resin), 7.9%. LC-MS (ESI^+): calculated for $C_{35}H_{56}N_8O_8$ $[M+H]^+$ *m/z:* 717.4; found: 717.5, $[M+2H]^{2+}$ *m/z:* 359.2; found: 359.3. (Figure S4). Compound **16c**: ^1H NMR (600 MHz, DMSO-d_6) δ 8.50 (d, *J* = 5.4 Hz, 1H), 8.02 (d, *J* = 7.8 Hz, 1H), 7.93 (d, *J* = 7.8 Hz, 1H), 7.75 (d, *J* = 7.2 Hz, 1H), 7.69 (s, 4H), 7.58 (d, *J* = 8.4 Hz, 1H), 7.46 (d, *J* = 7.2 Hz, 1H), 7.31 (d, *J* = 12.6 Hz, 2H), 7.25 – 7.22 (m, 3H), 7.18 (d, *J* = 7.2 Hz, 4H), 5.91 (d, *J* = 6.0 Hz, 1H), 4.60 (q, *J* = 7.8 Hz, 1H), 4.55 (dd, *J* = 9.0, 2.4 Hz, 1H), 4.42 (dd, *J* = 8.4, 2.4 Hz, 1H), 4.25 (q, *J* = 6.6 Hz, 1H), 4.21 (t, *J* = 6.0 Hz, 1H), 4.00 (q, *J* = 6.0 Hz, 1H), 3.76 (t, *J* = 7.2 Hz, 1H), 3.01 (dd, *J* = 13.2, 6.0 Hz, 1H), 2.83 – 2.78 (m, 4H), 1.93 – 1.84 (m, 4H), 1.72 – 1.64 (m, 1H), 1.58 – 1.45 (m, 8H), 1.38 – 1.32 (m, 1H), 1.19 – 1.13 (m, 1H), 0.86 (dd, *J* = 12.6, 6.6 Hz, 14H), 0.83 (d, *J* = 6.6 Hz, 5H), 0.74 (dd, *J* = 13.2, 7.2 Hz, 9H).

Compound **16d**: 3.9 mg (from 100 mg resin), 6.6%. LC-MS (ESI^+): calculated for $C_{37}H_{57}ClN_8O_7$ $[M+H]^+$ *m/z:* 761.4; found: 761.5, $[M+2H]^{2+}$ *m/z:* 381.2; found: 381.3. (Figure S5). Compound **16d**: ^1H NMR (600 MHz, DMSO-d_6) δ 11.04 (s, 1H), 8.26 (d, *J* = 6.6 Hz, 1H), 8.09 (d, *J* = 8.4 Hz, 1H), 7.91 (d, *J* = 9.0 Hz, 1H), 7.82 (d, *J* = 8.4 Hz, 1H), 7.71 – 7.64 (m, 4H), 7.60 (d, *J* = 6.0 Hz, 1H), 7.56 (s, 1H), 7.32 (d, *J* = 8.4 Hz, 1H), 7.21 (s, 1H), 7.04 (d, *J* = 9.0 Hz, 1H), 4.51 (q, *J* = 7.8 Hz, 1H), 4.36 (q, *J* = 5.4 Hz, 1H), 4.21 (t, *J* = 8.4 Hz, 1H), 4.17 (q, *J* = 9.0 Hz, 1H), 4.15 – 4.11 (m, 1H), 3.89 – 3.85 (m, 1H), 3.85 – 3.80 (m, 1H), 3.07 (dd, *J* = 13.8, 7.8 Hz, 1H), 2.90 (dd, *J* = 13.8, 6.0 Hz, 1H), 2.80 – 2.76 (m, 2H), 1.91 – 1.85 (m, 2H), 1.84 – 1.77 (m, 1H), 1.65 – 1.59 (m, 1H), 1.50 – 1.46 (m, 6H), 1.29 – 1.23 (m, 1H), 1.18 – 1.13 (m, 1H), 1.01 (d, *J* = 6.0 Hz, 3H), 0.89 (d, *J* = 5.4 Hz, 3H), 0.84 – 0.81 (m, 10H), 0.57 – 0.49 (m, 7H).

4、LCMS traces of Nicrophorusamides A and analogues

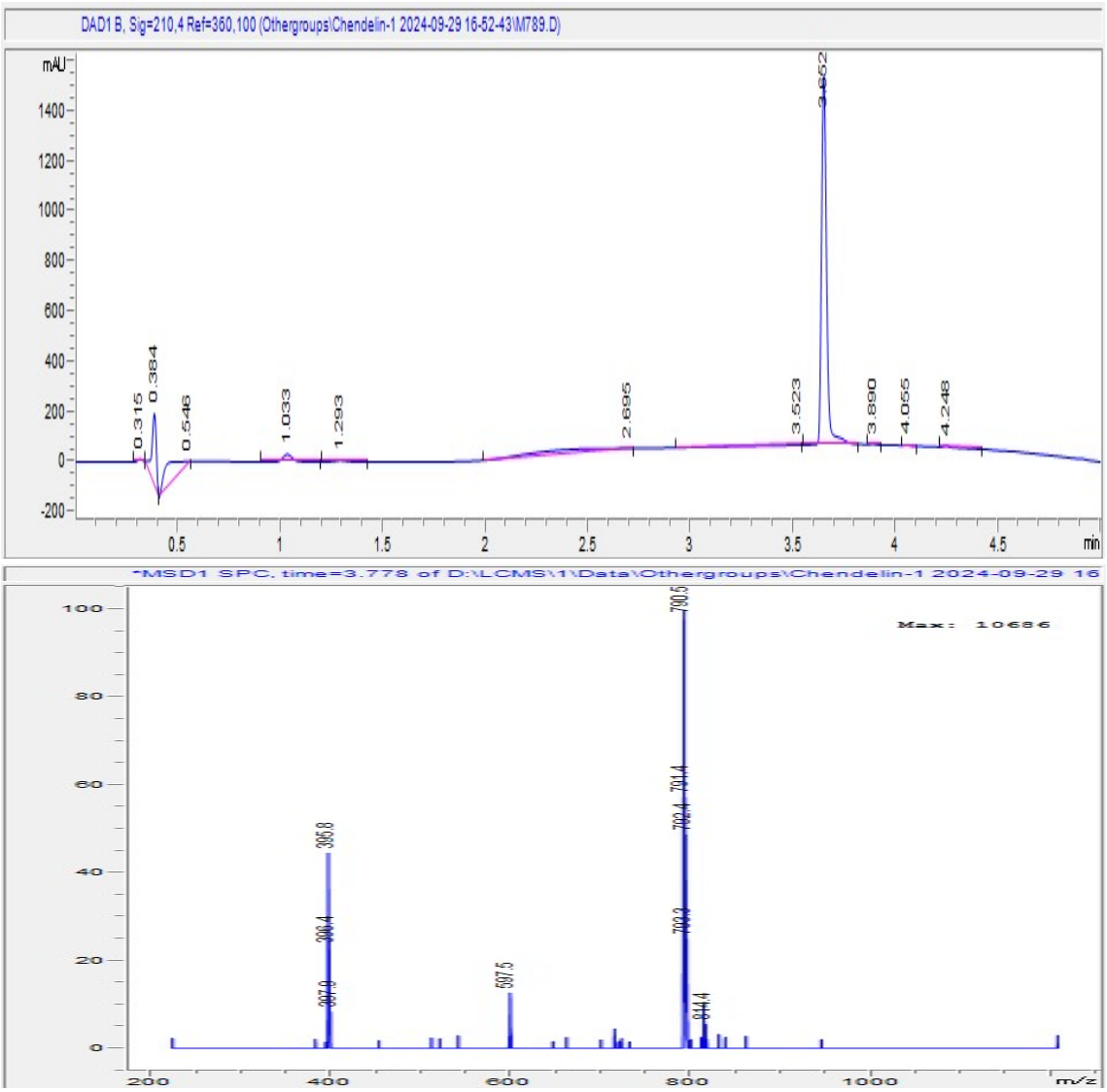


Figure S1. Analytical UPLC analysis UV trace and ESI-MS of purified compoud **15**. Gradient: 5-95 % MeCN over 5 min at a flow rate of 0.4 mL/min. MS (ESI$^+$) calcd. for $C_{37}H_{56}ClN_9O_8$ $[M+H]^+$ *m/z:* 790.4; found: 790.5, $[M+2H]^{2+}$ *m/z:* 395.7; found: 395.8.

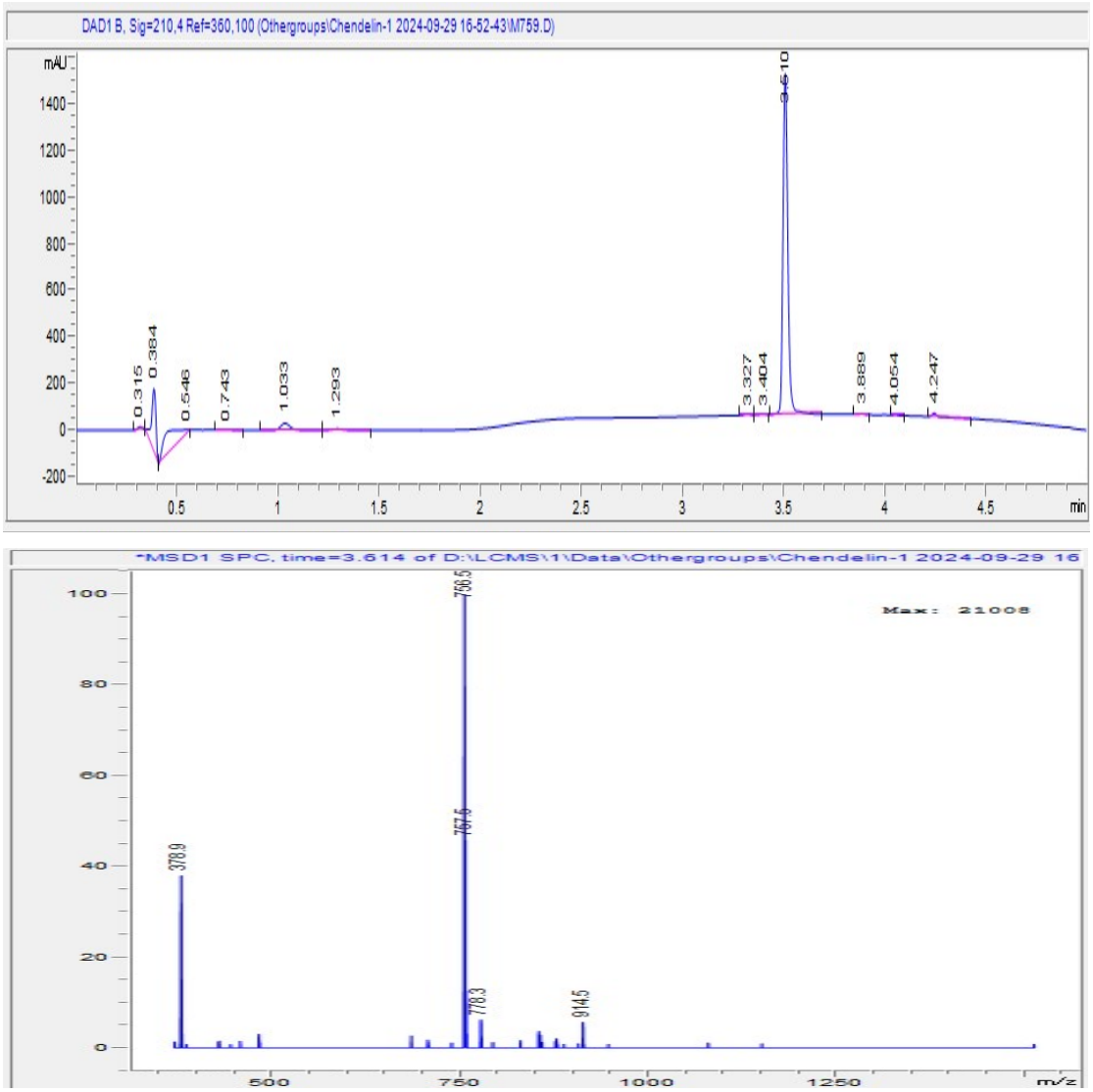


Figure S2. Analytical UPLC analysis UV trace and ESI-MS of purified compoud **16a**. Gradient: 5-95 % MeCN over 5 min at a flow rate of 0.4 mL/min. MS (ESI^+) calcd. for $C_{37}H_{57}N_9O_8$ $[M+H]^+$ *m/z:* 756.4 ; found: 756.5, $[M+2H]^{2+}$ *m/z:* 378.7; found: 378.9.

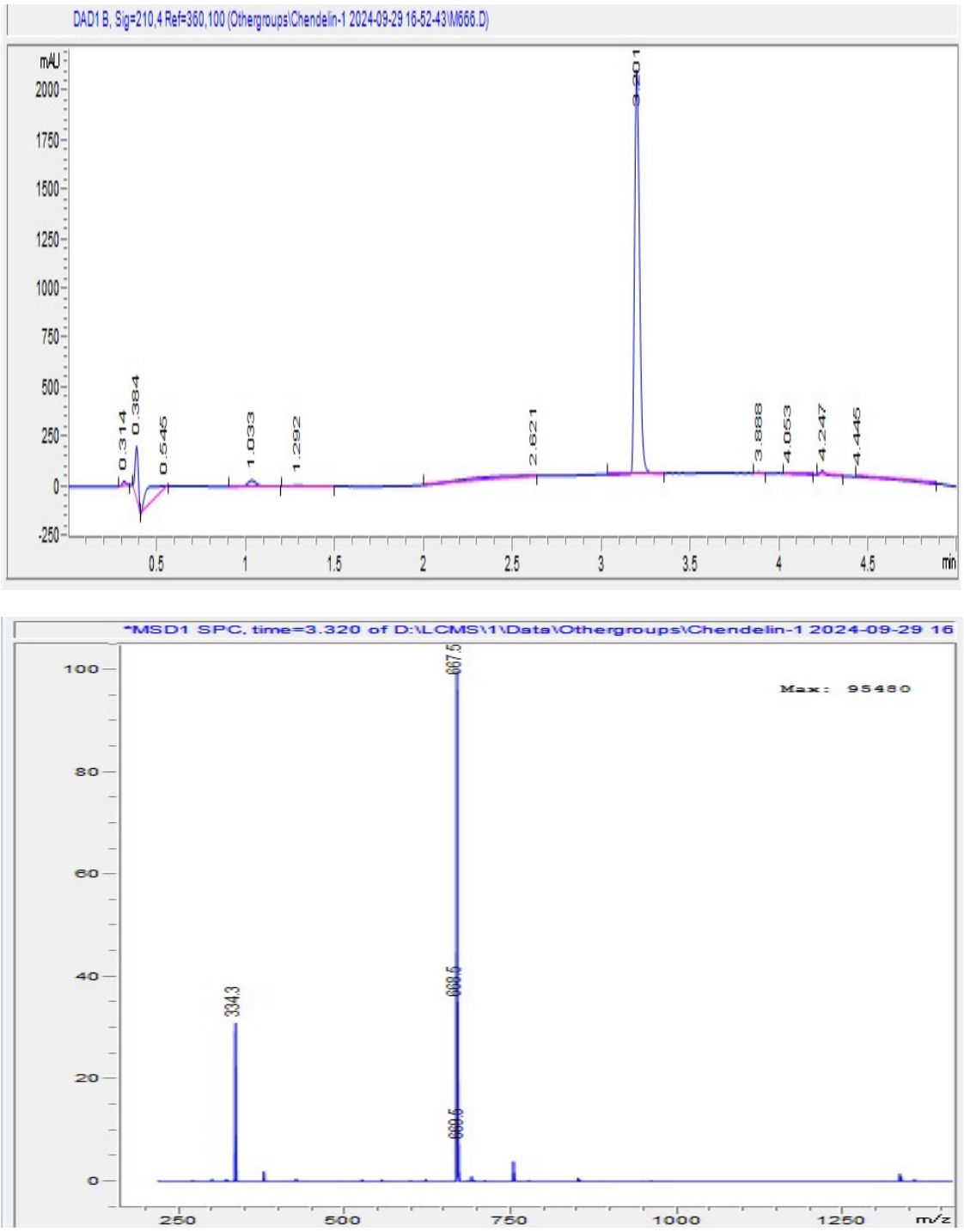


Figure S3. Analytical UPLC analysis UV trace and ESI-MS of purified compoud **16b**. Gradient: 5-95 % MeCN over 5 min at a flow rate of 0.4 mL/min. MS (ESI^+) calcd. for $C_{31}H_{54}N_8O_8$ $[M+H]^+$ m/z: 667.4; found: 667.5, $[M+2H]^{2+}$ m/z: 334.2; found: 334.3.

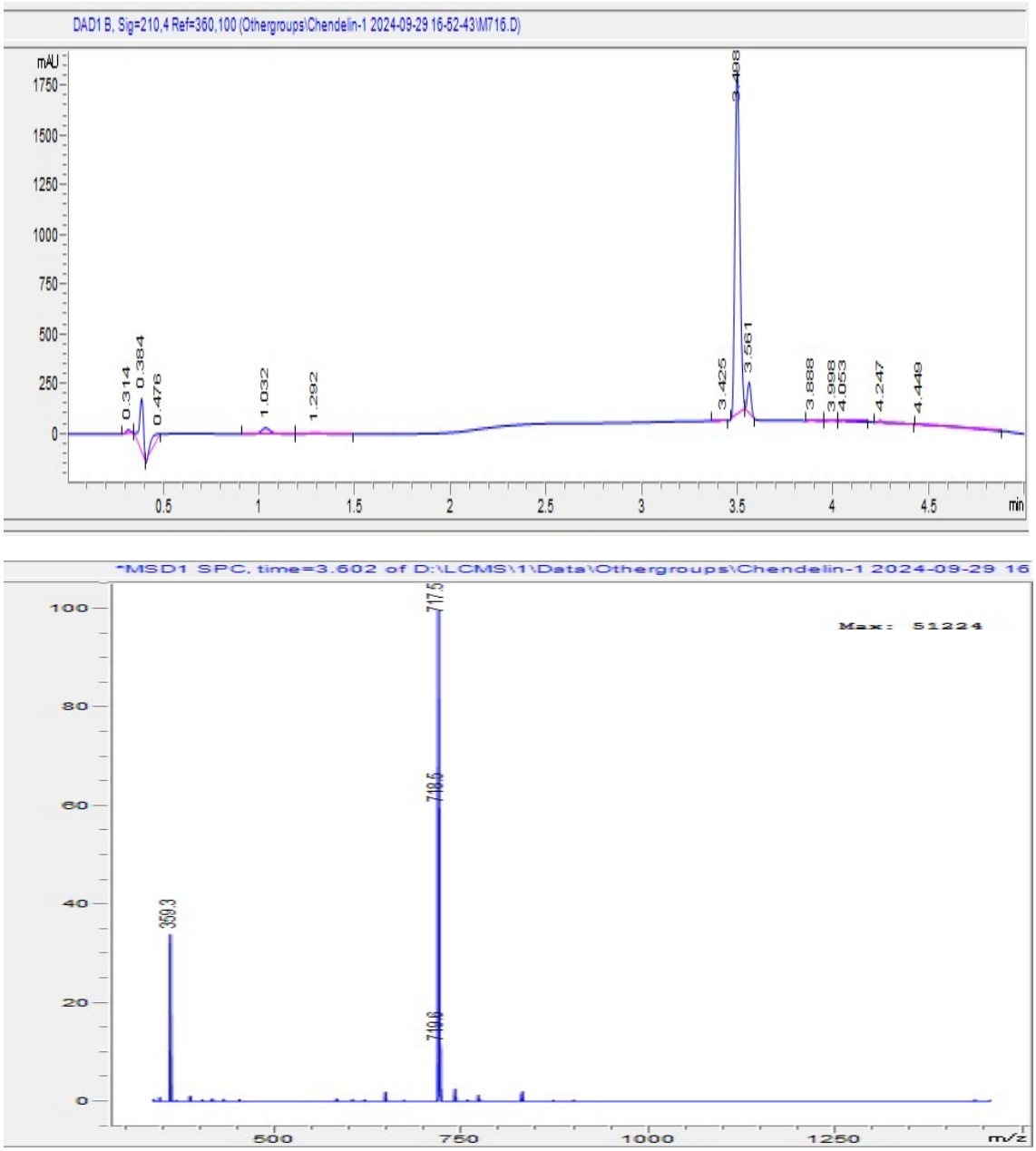


Figure S4. Analytical UPLC analysis UV trace and ESI-MS of purified compoud **16c**. Gradient: 5-95 % MeCN over 5 min at a flow rate of 0.4 mL/min. MS (ESI^+) calcd. for $C_{35}H_{56}N_8O_8$ $[M+H]^+$ *m/z:* 717.4; found: 717.5, $[M+2H]^{2+}$ *m/z:* 359.2; found: 359.3.

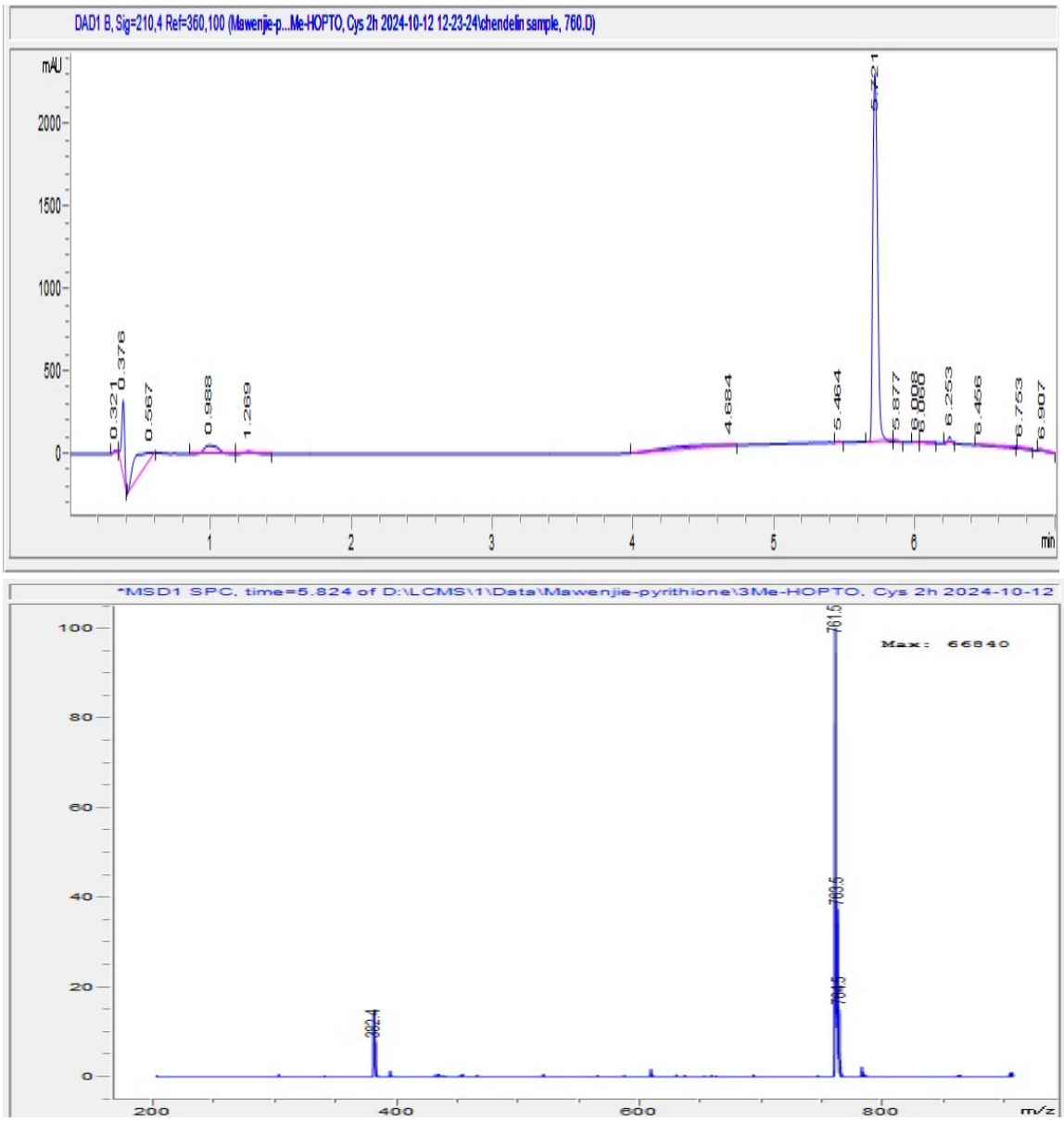
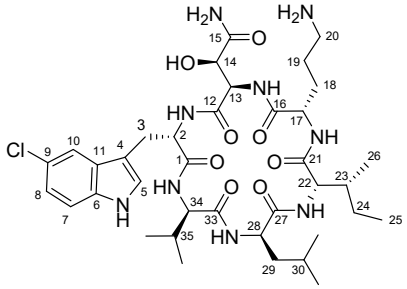


Figure S5. Analytical UPLC analysis UV trace and ESI-MS of purified compoud **16d**. Gradient: 5-95 % MeCN over 7 min at a flow rate of 0.4 mL/min. MS (ESI^+) calcd. for $C_{37}H_{57}ClN_8O_7$ $[M+H]^+$ *m/z:* 761.4; found: 761.5, $[M+2H]^{2+}$ *m/z:* 381.2; found: 381.3.

5、NMR Data Comparison between Natural Nicrophorusamide A and Synthetic Compoud 15.

Table 1. ^{13}C NMR[a] comparison between natural nicrophorusamide A and synthetic compoud **15**.



Nicrophorusamide A (15)

Carbon No.	^{13}C NMR		$\Delta\delta^{13}C$
	natural / δ_{A1} (ppm)	synthetic / δ_{A2} (ppm)	$\delta A1$- $\delta A2$
1	171.1	171.1	0.0
2	53.5	53.6	-0.1
3	26.7	26.9	-0.2
4	109.8	109.8	0.0
5	125.4	125.5	-0.1
6	134.5	134.5	0.0
7	112.7	112.8	-0.1
8	120.7	120.7	0.0
9	122.9	122.9	0.0
10	117.5	117.5	0.0
11	128.4	128.4	0.0
12	169.4	169.4	0.0
13	55.8	55.8	0.0
14	70.8	70.8	0.0
15	173.2	173.3	-0.1
16	170.6	170.7	-0.1

17	53.1	53.1	0.0
18	26.8	26.8	0.0
19	23.7	23.8	-0.1
20	38.4	38.4	0.0
21	171.7	171.7	0.0
22	56.5	56.4	0.1
23	36.3	36.3	0.0
24	25.6	25.6	0.0
25	11.3	11.4	-0.1
26	14.5	14.5	0.0
27	172.1	172.1	0.0
28	51.5	51.6	-0.1
29	39.6	overlap	-
30	24.3	24.4	-0.1
31	22.7	22.8	-0.1
32	21.8	21.9	-0.1
33	170.4	170.4	0.0
34	60.4	60.4	0.0
35	29.1	29.1	0.0
36	18.5	18.6	-0.1
37	18.8	18.9	-0.1

[a]All chemical shifts are reported in ppm. All spectra were measured in DMSO-d_6 and referenced to the residual solvent peak at δ_H =2.50 ppm and δ_C = 39.52 ppm as done in the original isolation report[1]. ^{1}H-NMR spectrum was recorded at 400 MHz. ^{13}C-NMR spectrum was recorded at 150 MHz.

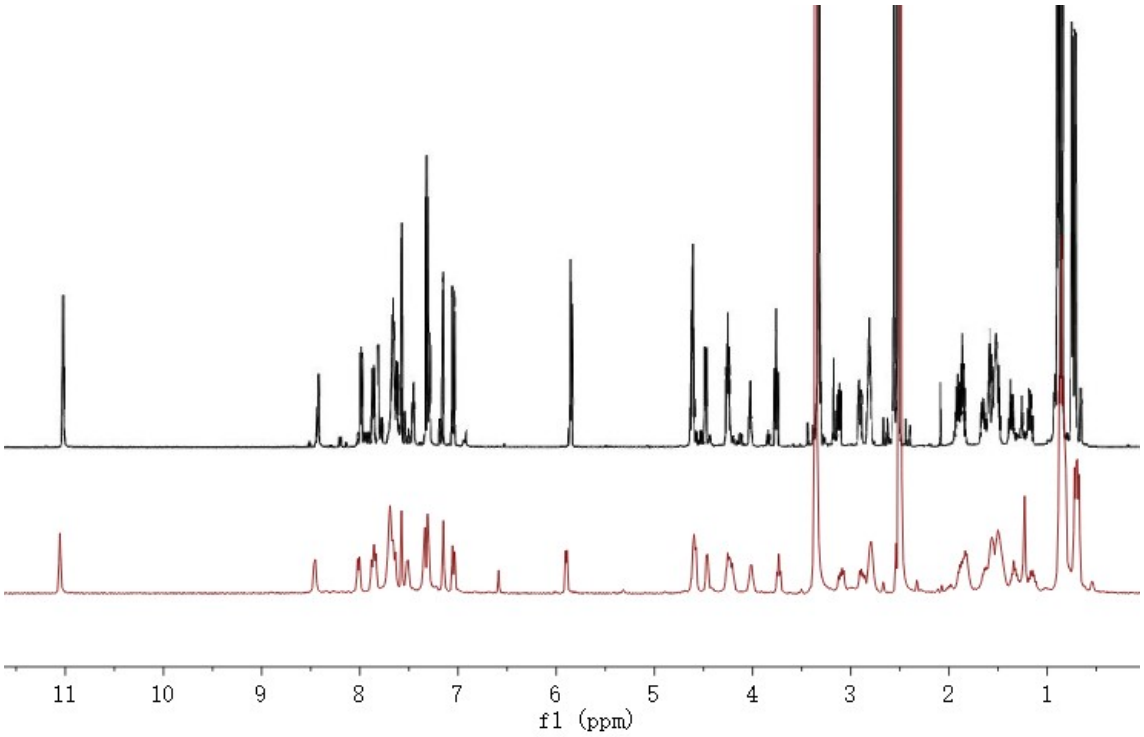


Figure S6. Comparison of ^{1}H NMR (DMSO-d_6, 400 MHz) spectra of synthetic Nicrophorusamide A (red) and Nicrophorusamide A (black, screenshot from supporting information of Nicrophorusamide A's isolation article published in 2017[1])

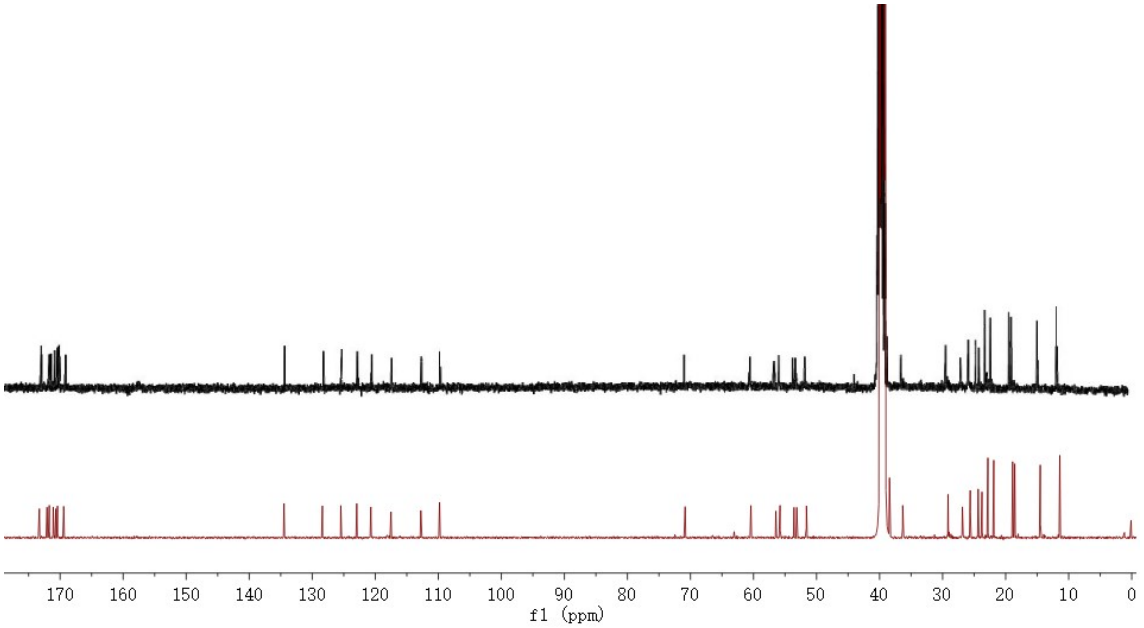


Figure S7. Comparison of ^{13}C NMR (DMSO-d_6, 150 MHz) spectra of synthetic Nicrophorusamide A (red) and Nicrophorusamide A (black, screenshot from supporting information of Nicrophorusamide A's isolation article published in 2017[1]).

6、NMR Spectra

6.1 ^{1}H and ^{13}C NMR spectra of building blocks and intermediates

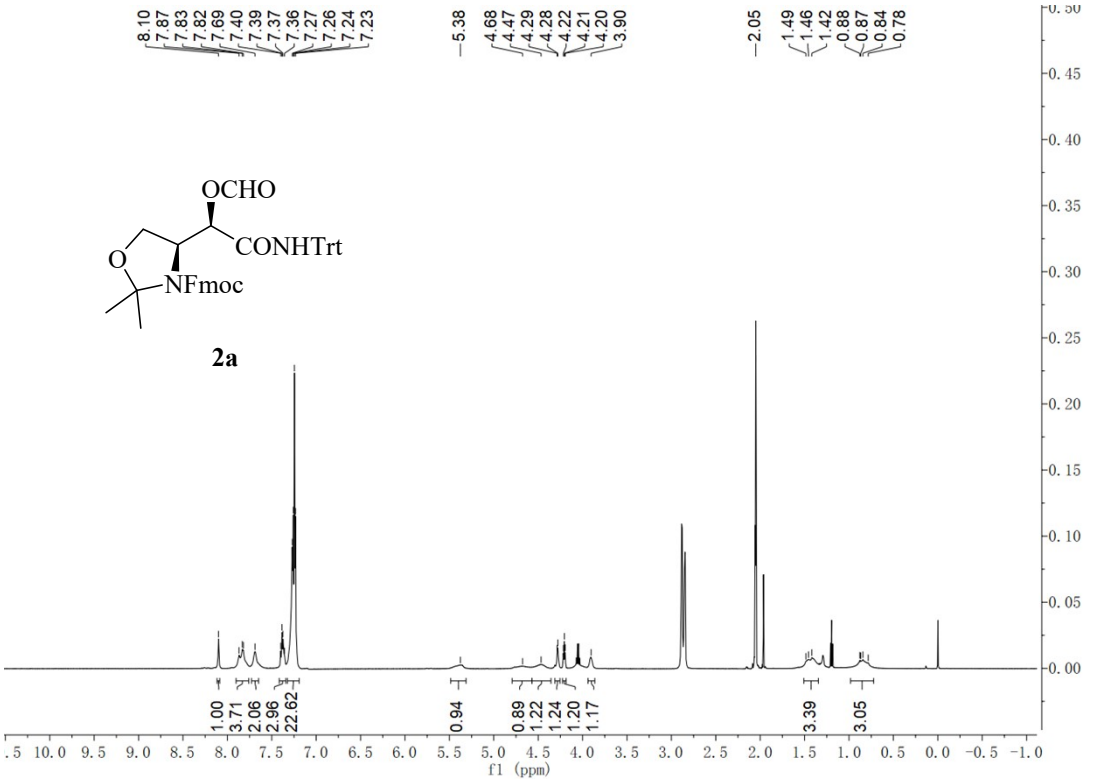


Figure S8. ^{1}H NMR spectrum of compound **2a.**

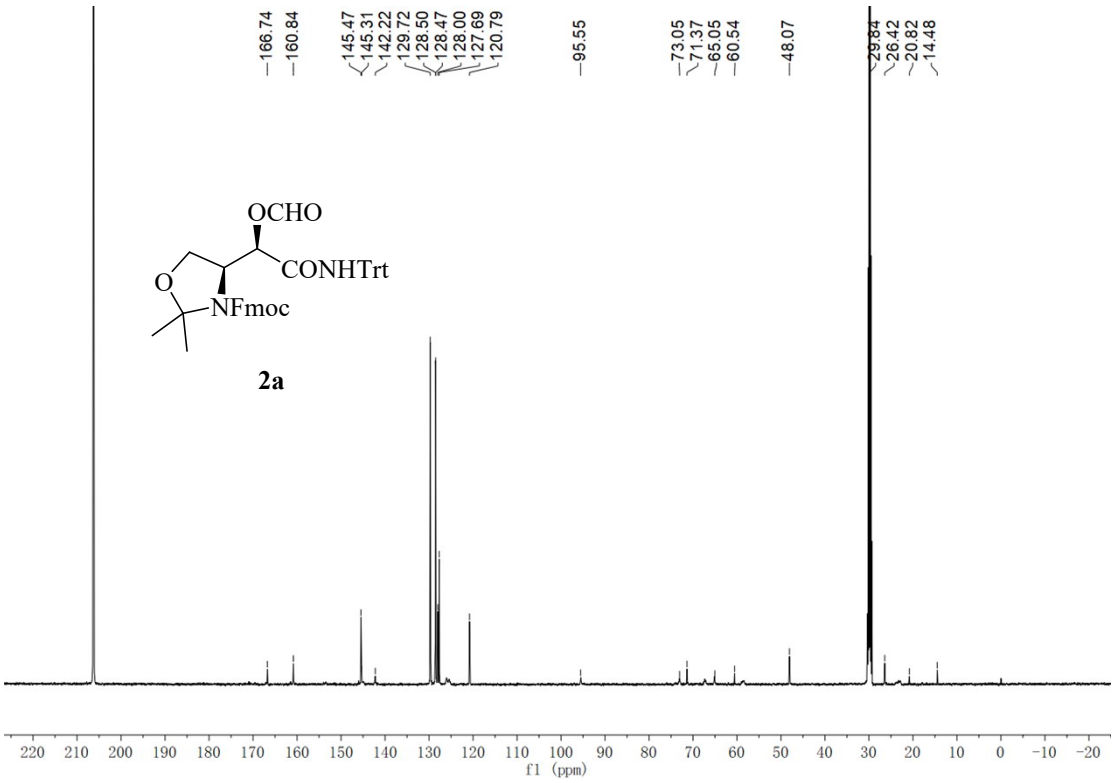


Figure S9. ^{13}C NMR spectrum of compound **2a.**

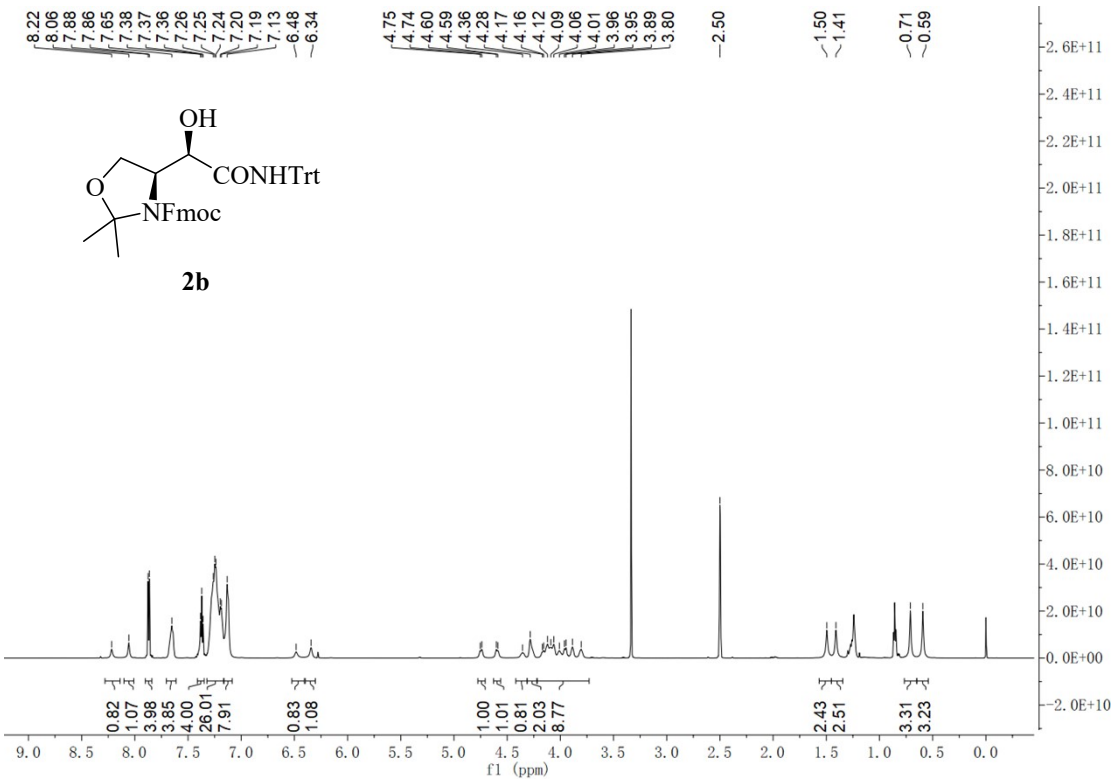


Figure S10. ^{1}H NMR spectrum of compound **2b.**

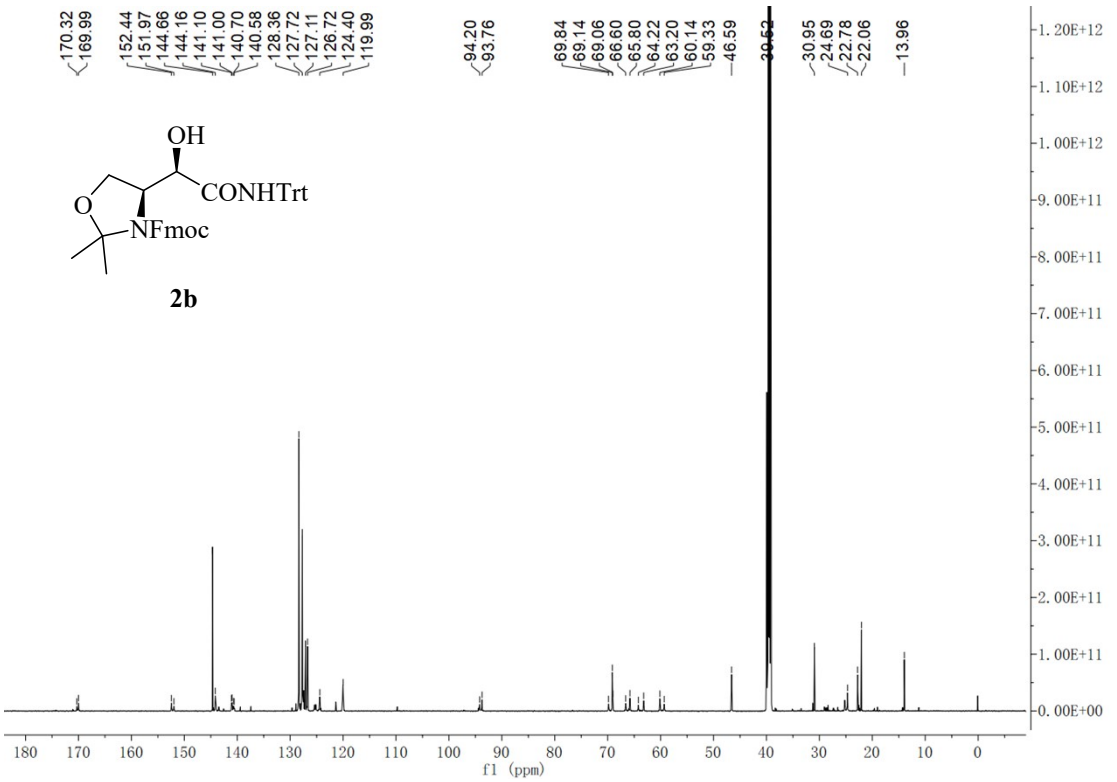


Figure S11. ^{13}C NMR spectrum of compound **2b.**

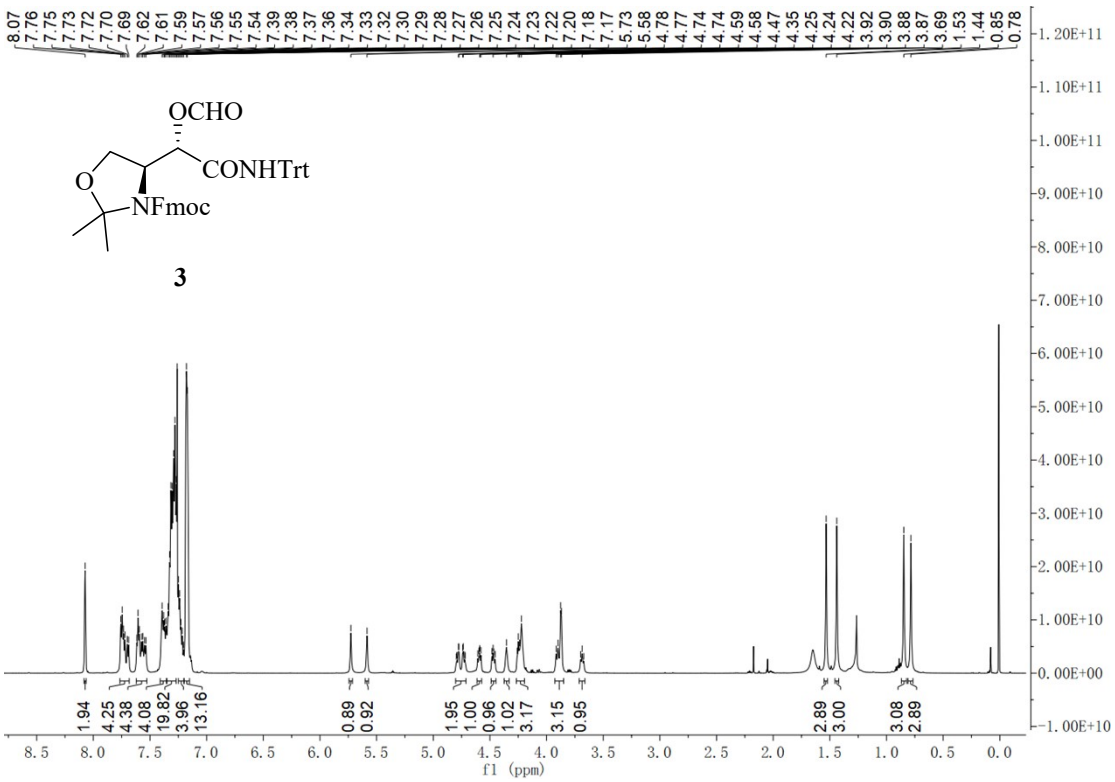


Figure S12. ^{1}H NMR spectrum of compound **3.**

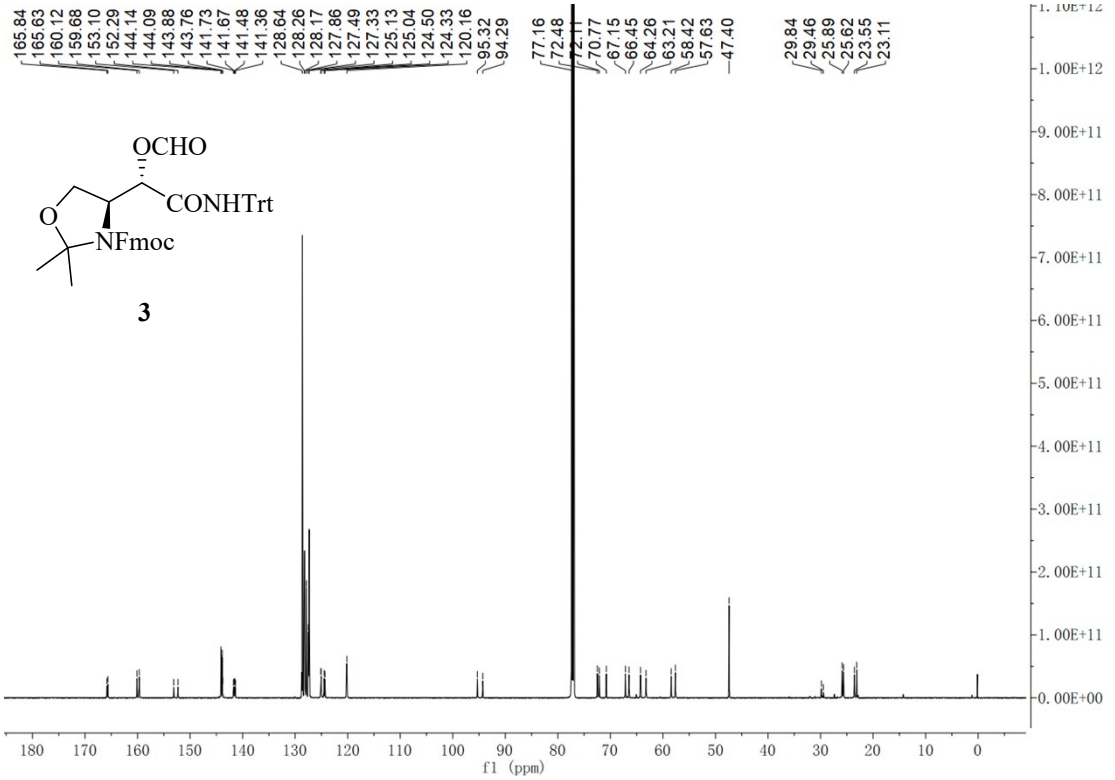


Figure S13. ^{13}C NMR spectrum of compound **3.**

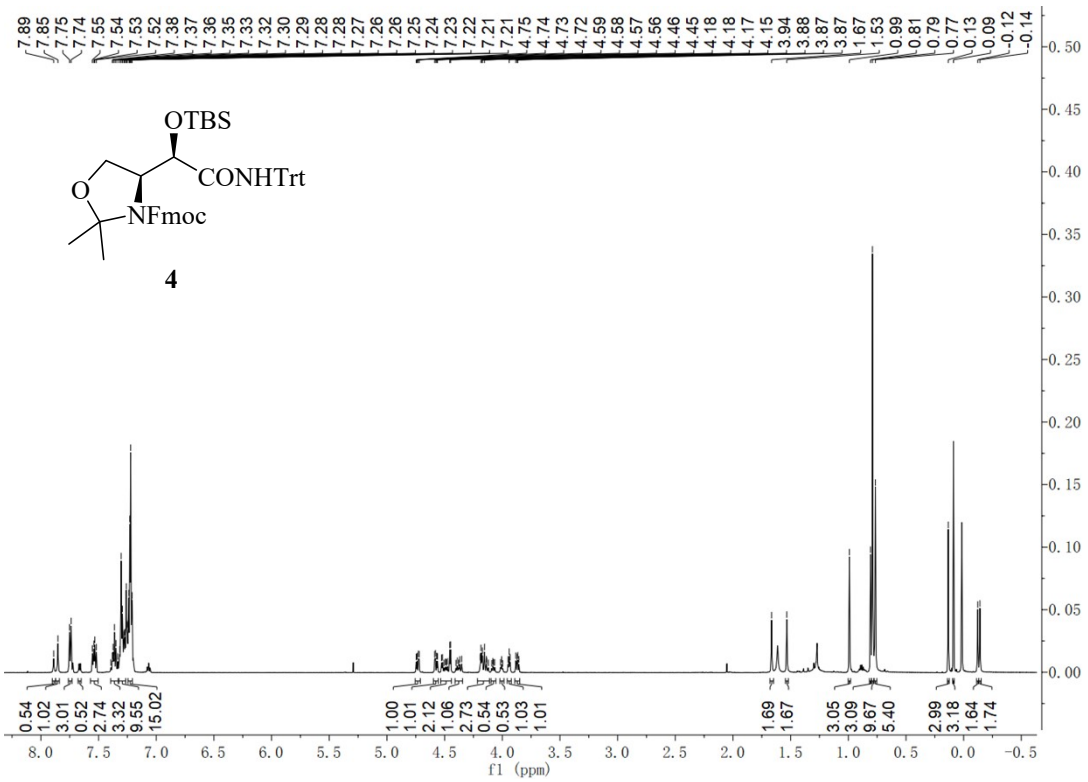


Figure S14. ^{1}H NMR spectrum of compound **4.**

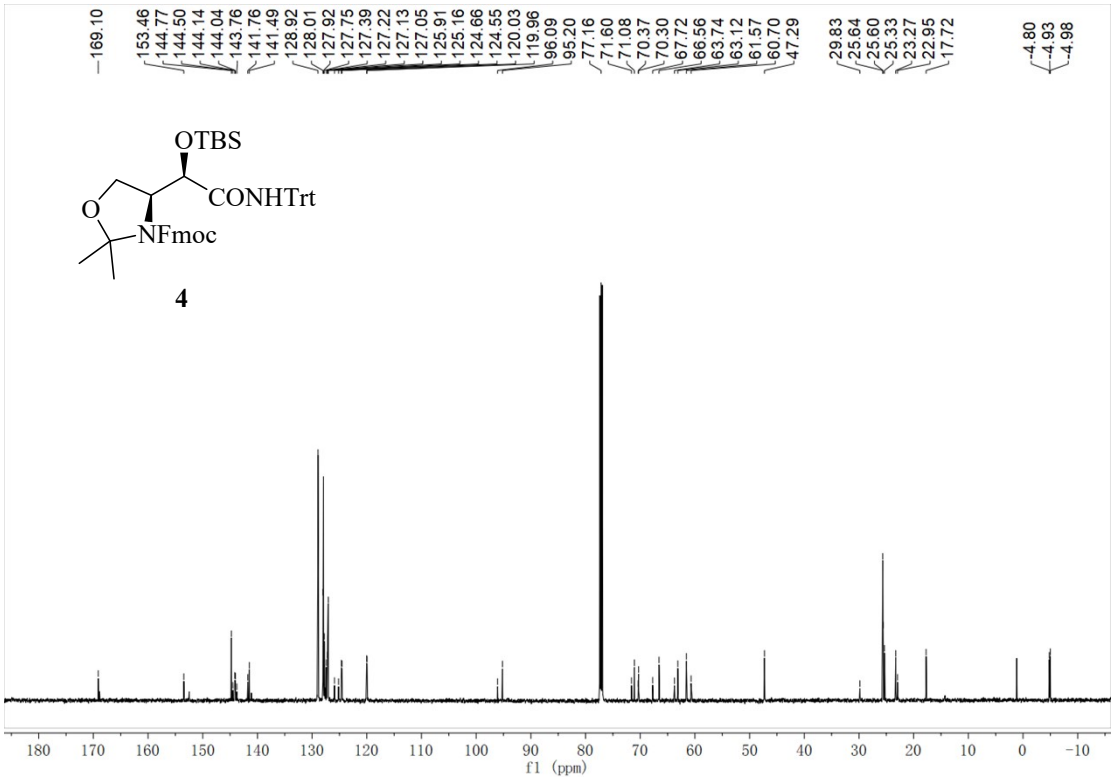


Figure S15. ^{13}C NMR spectrum of compound **4.**

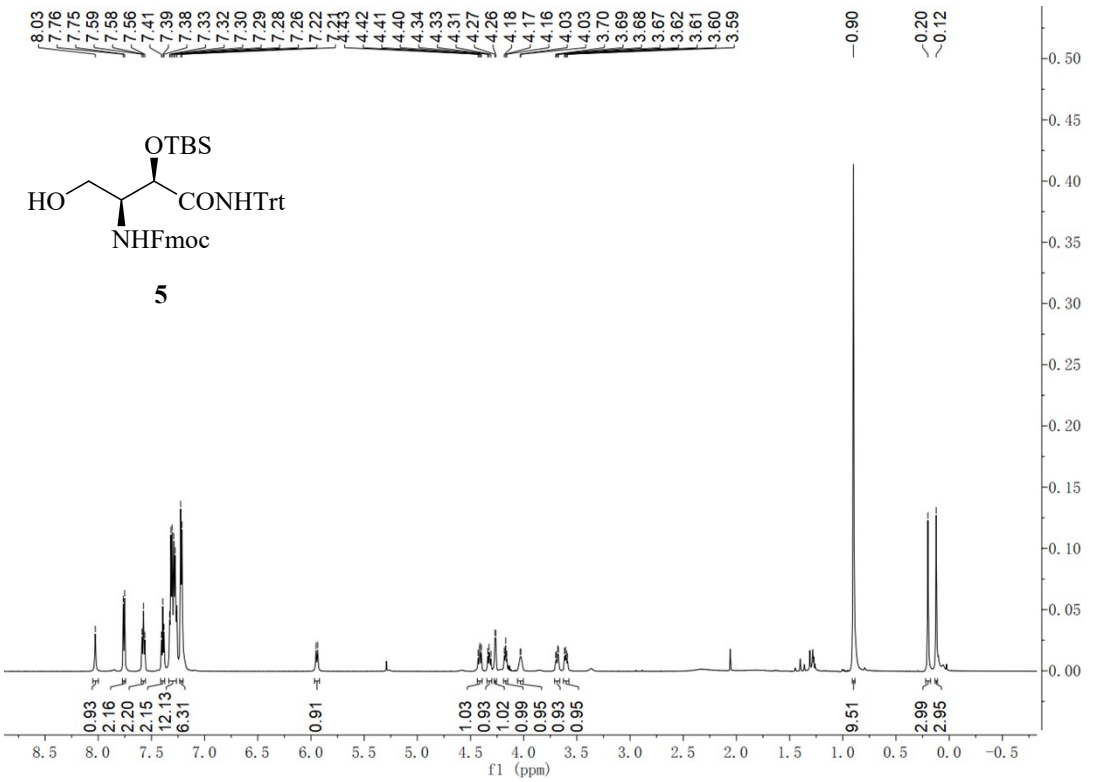


Figure S16. ^{1}H NMR spectrum of compound **5.**

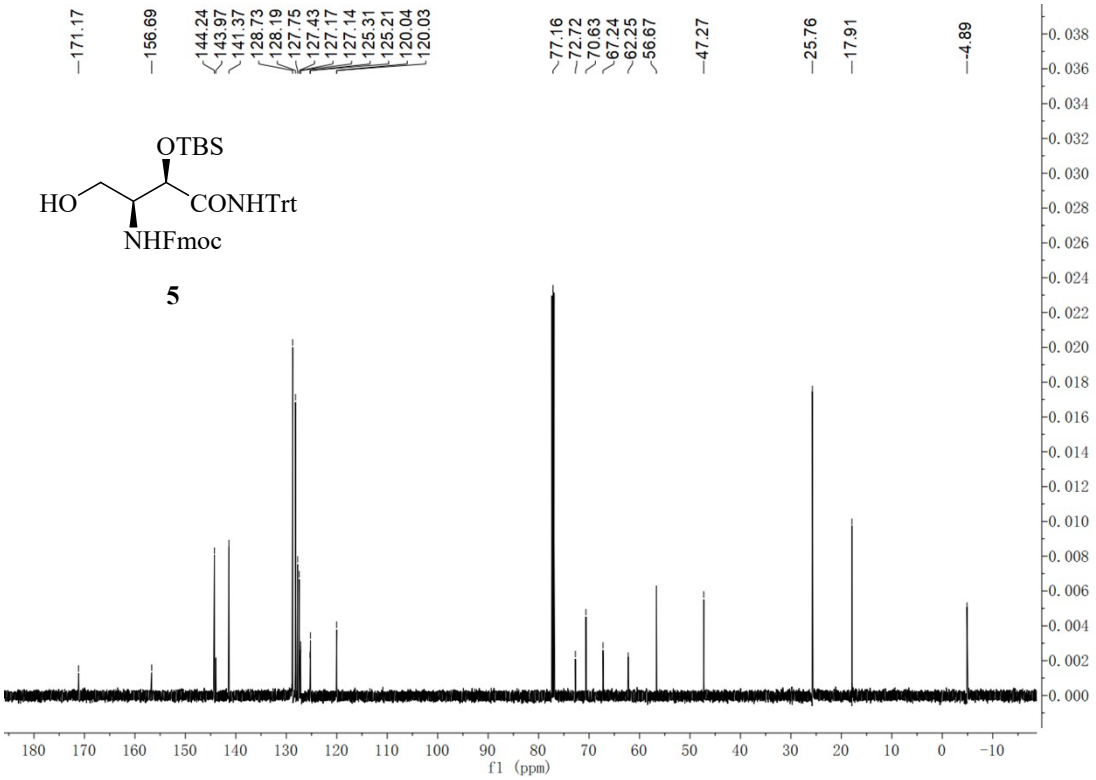


Figure S17. ^{13}C NMR spectrum of compound **5.**

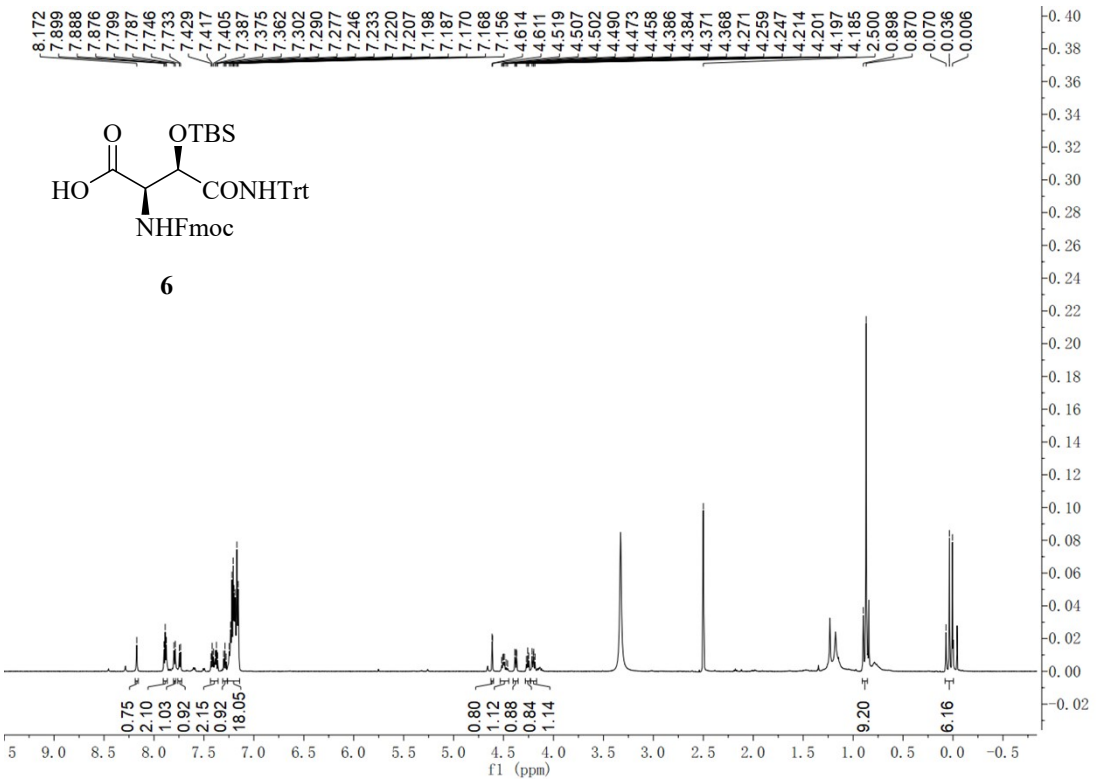


Figure S18. ^{1}H NMR spectrum of compound **6.**

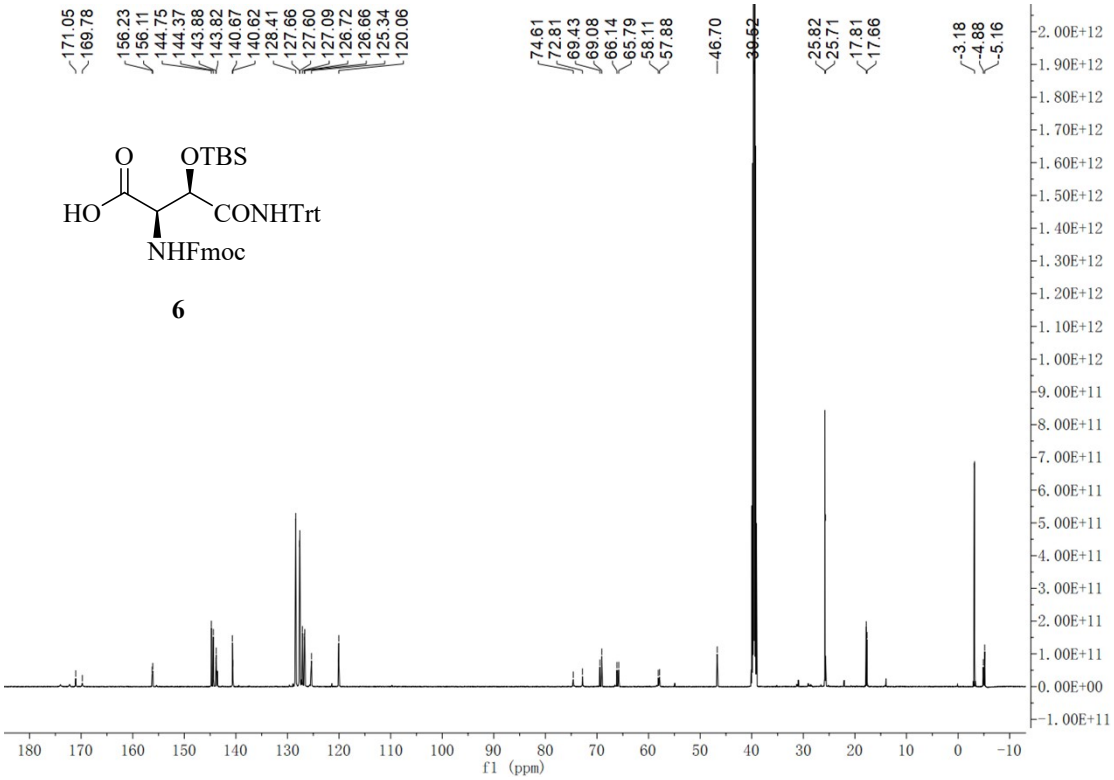


Figure S19. ^{13}C NMR spectrum of compound **6.**

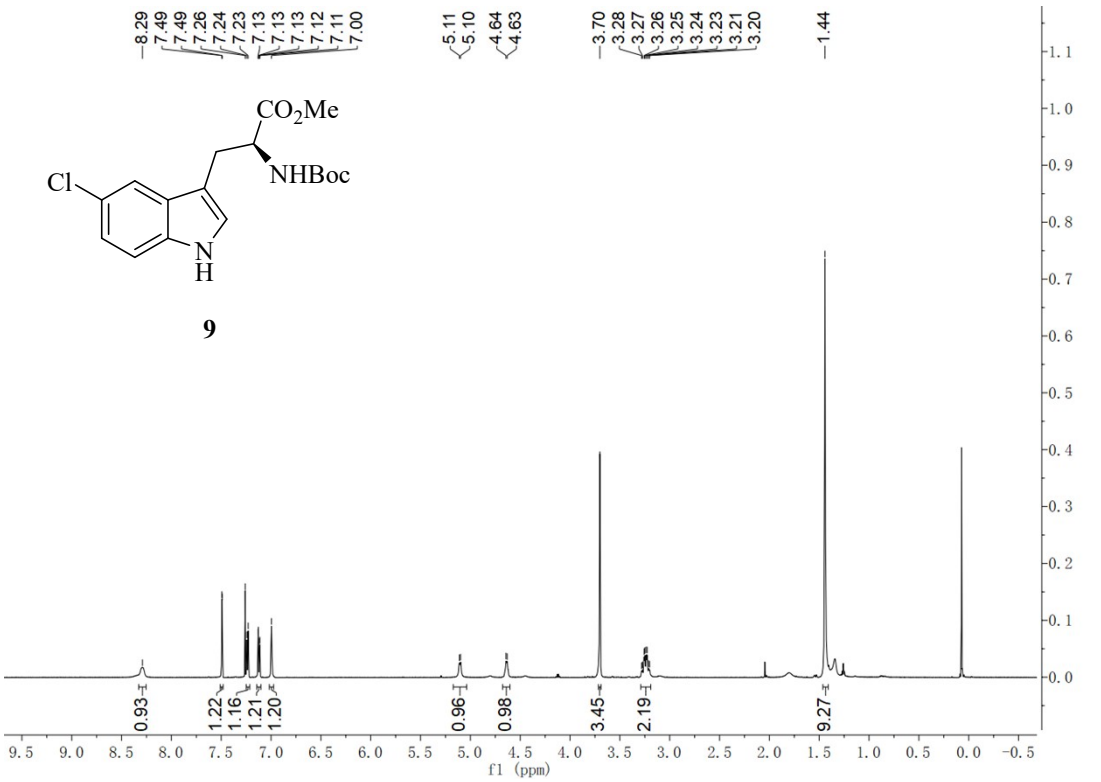


Figure S20. ^{1}H NMR spectrum of compound **9.**

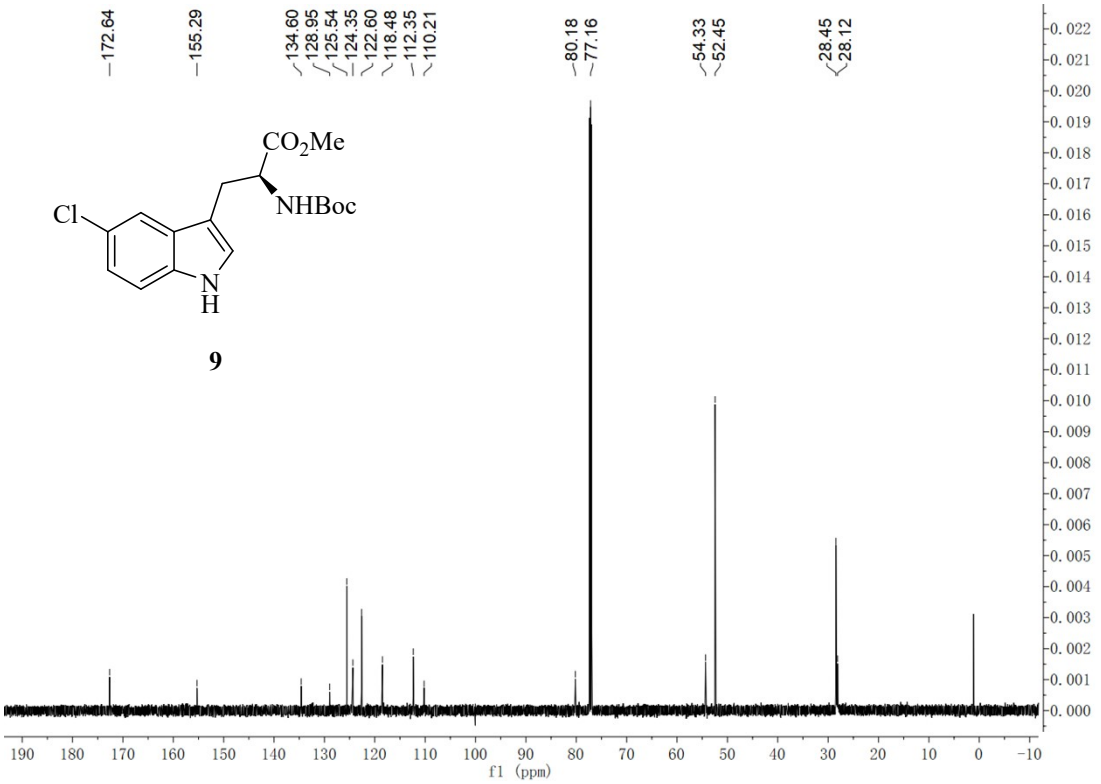


Figure S21. ^{13}C NMR spectrum of compound **9.**

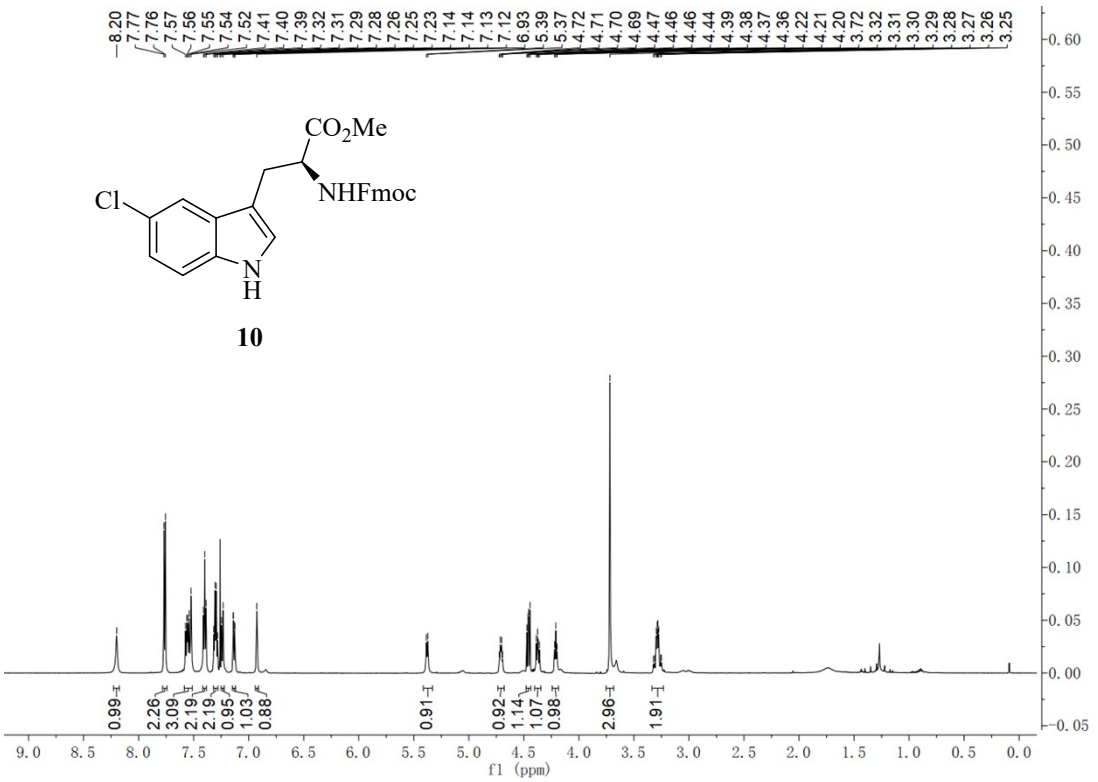


Figure S22. ^{1}H NMR spectrum of compound **10.**

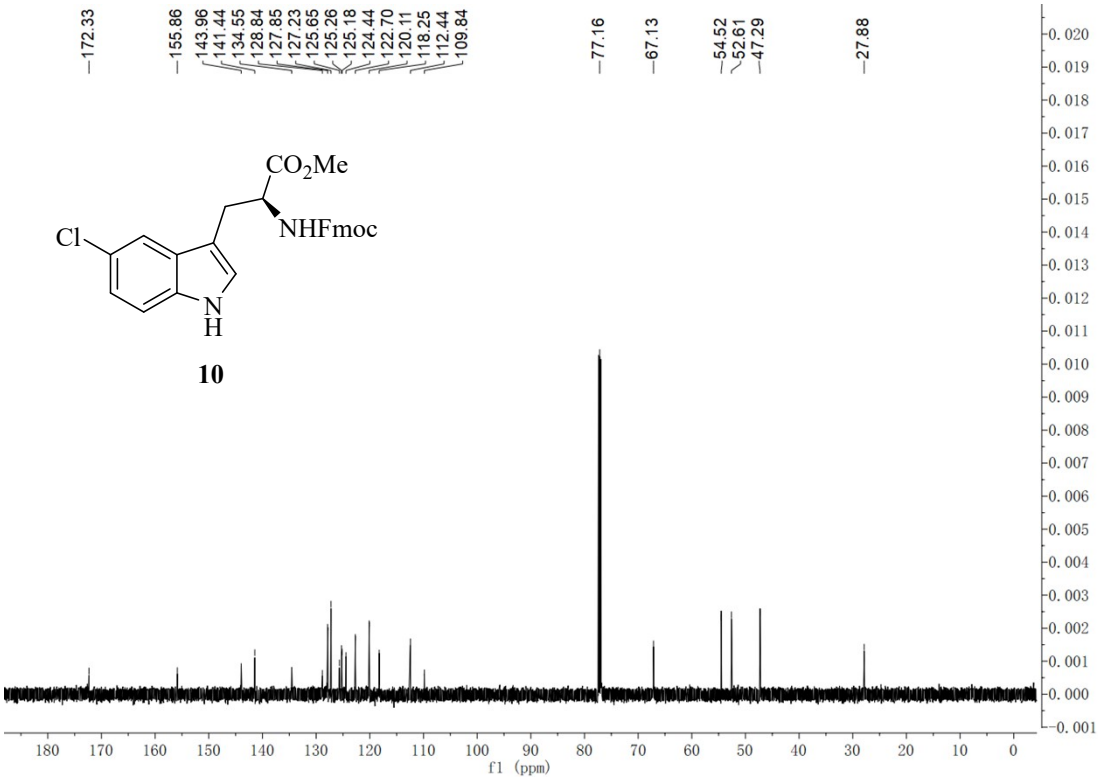


Figure S23. ^{13}C NMR spectrum of compound **10.**

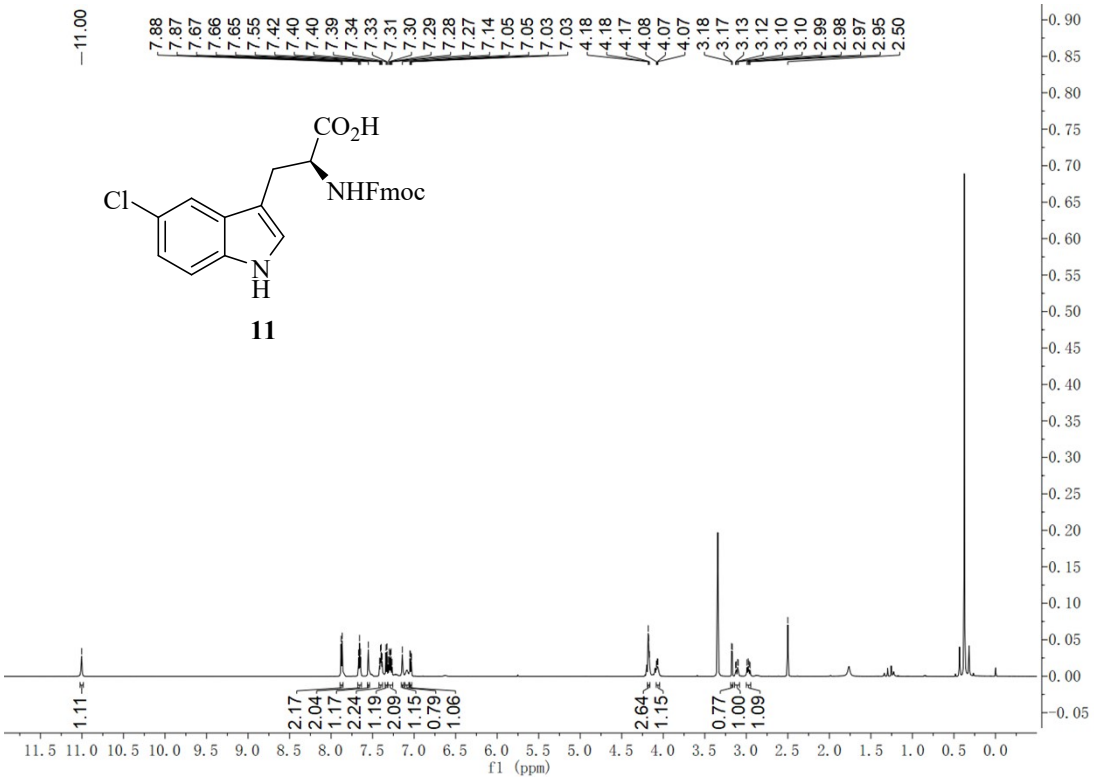


Figure S24. ^{1}H NMR spectrum of compound **11.**

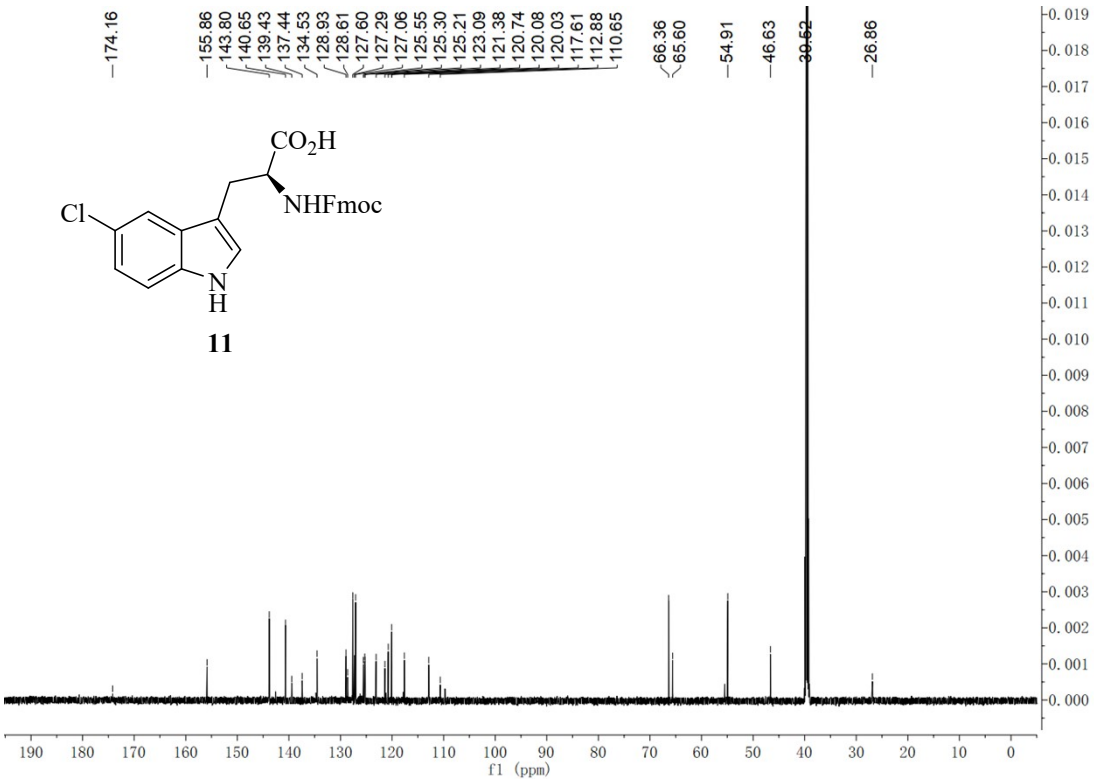


Figure S25. ^{13}C NMR spectrum of compound **11.**

6.2 ^{1}H and ^{13}C NMR spectra of synthetic Nicrophorusamides A

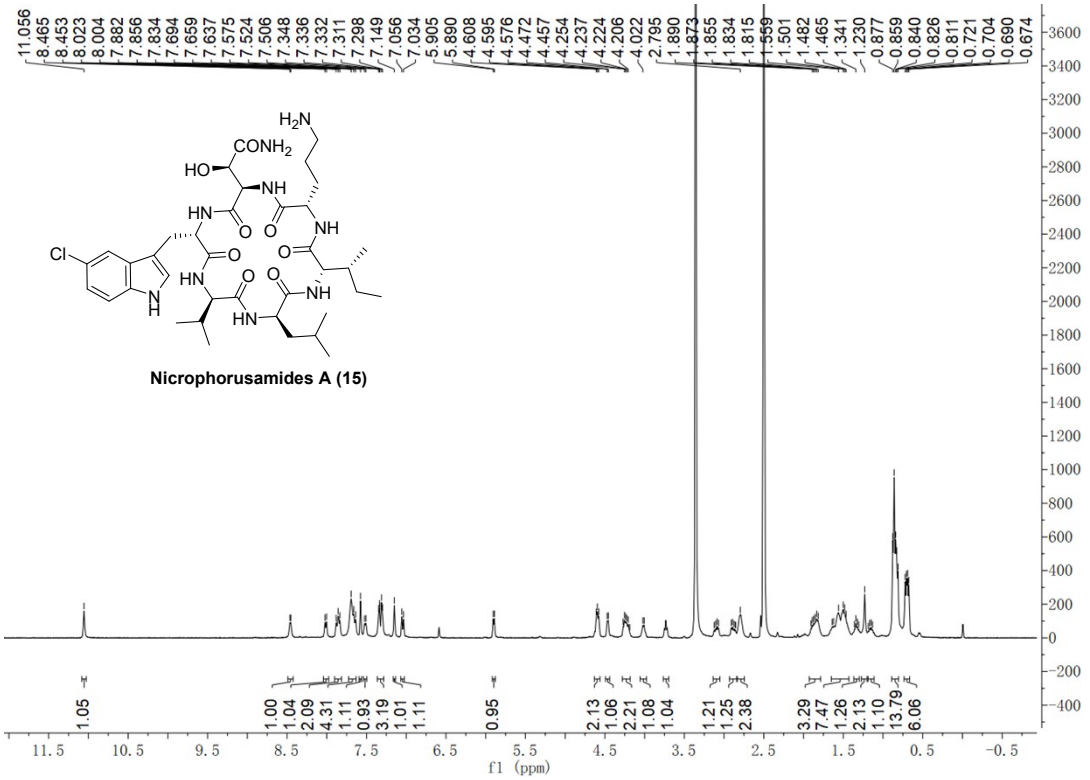


Figure S26. ^{1}H NMR spectrum of Nicrophorusamides A (compound **15**) .

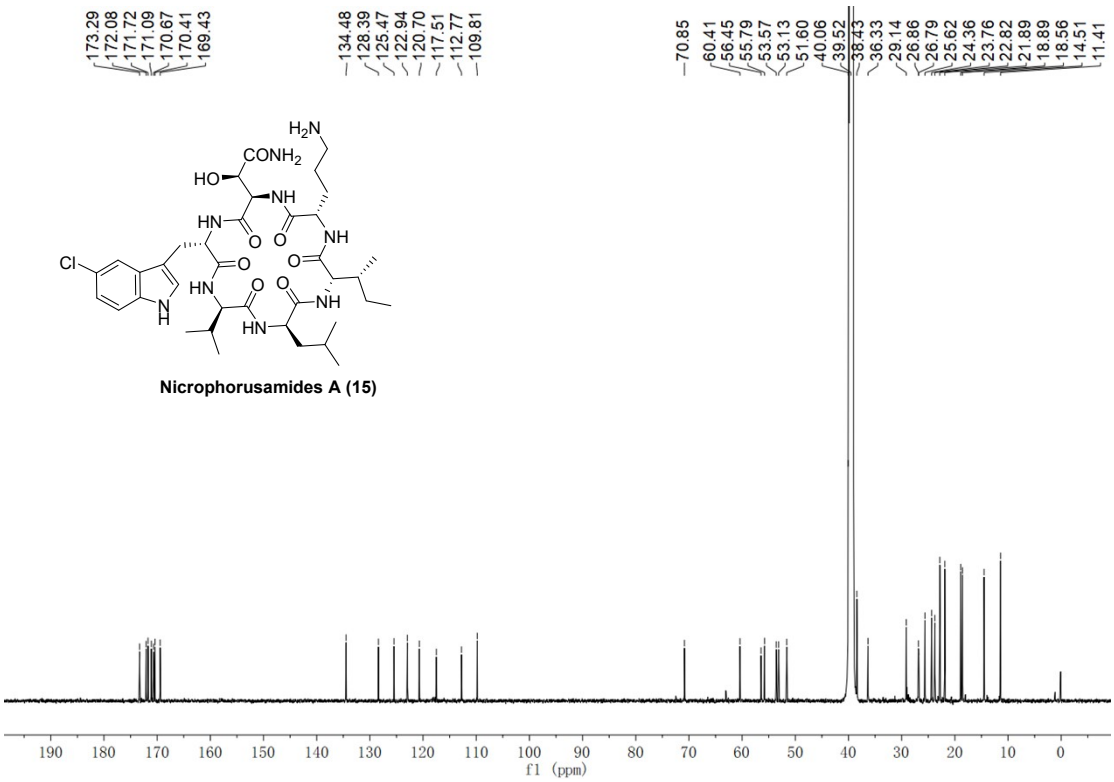


Figure S27. ^{13}C NMR spectrum of Nicrophorusamides A (compound **15**

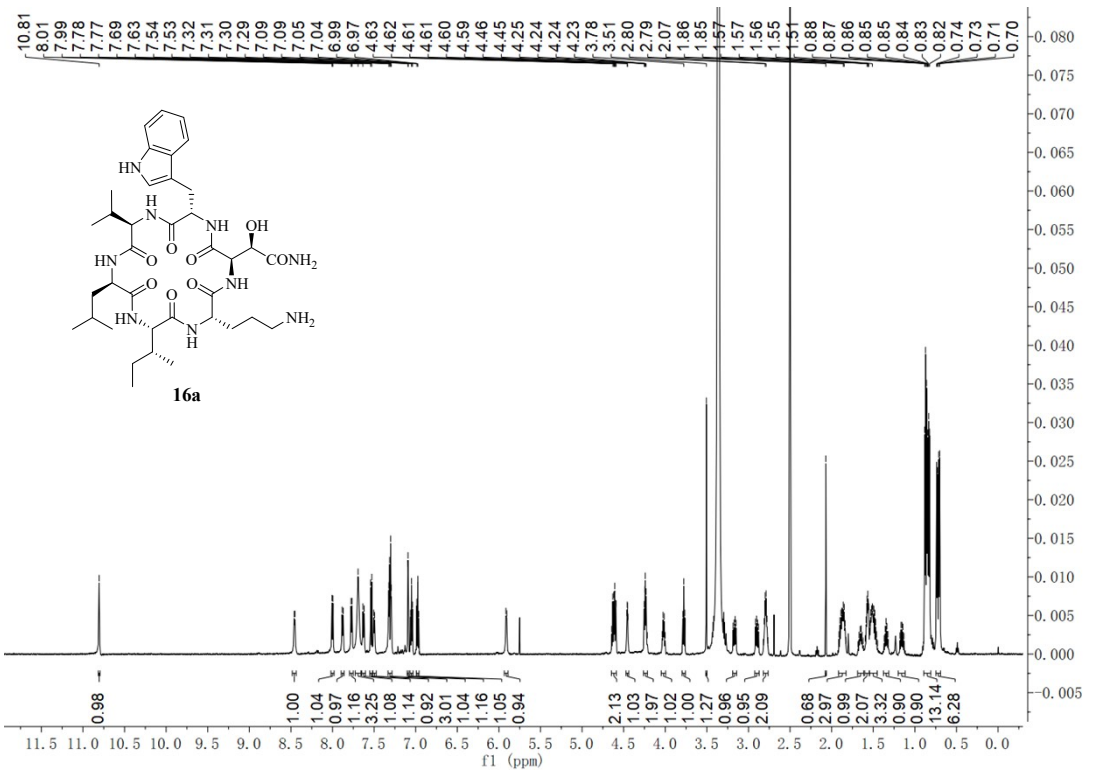


Figure S28. ^{1}H NMR spectrum of compound **16a**.

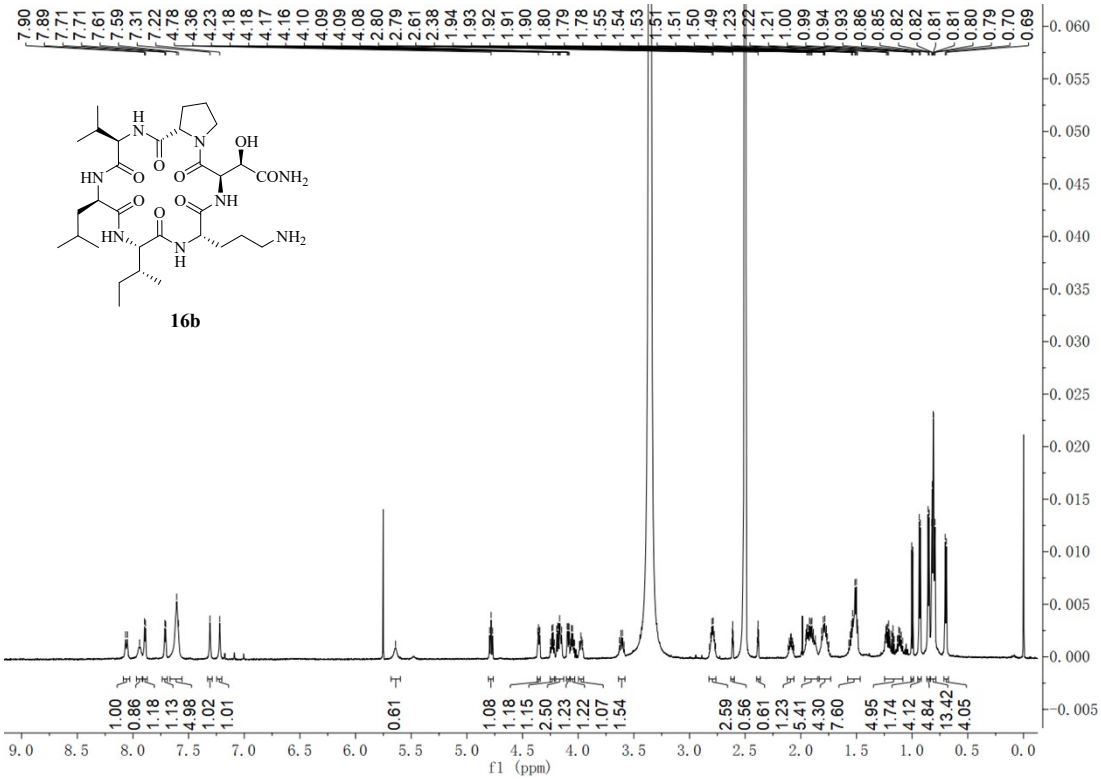


Figure S29. ^{1}H NMR spectrum of compound **16b**.

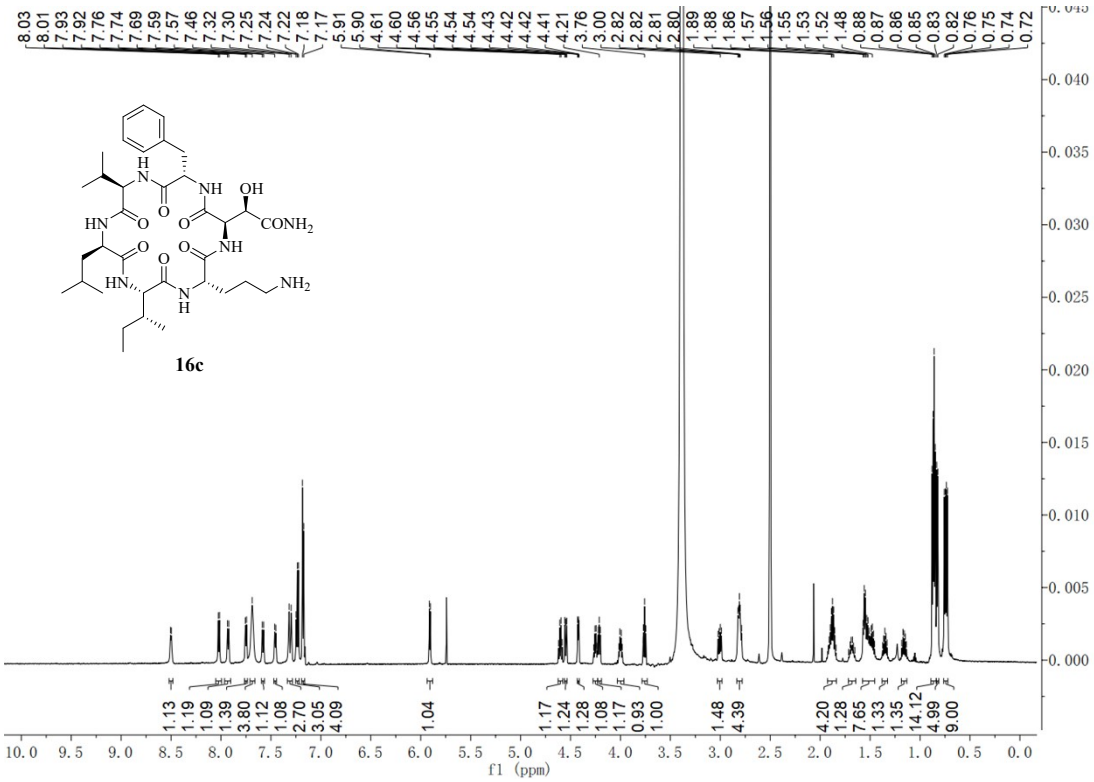


Figure S30. ^{1}H NMR spectrum of compound **16c**.

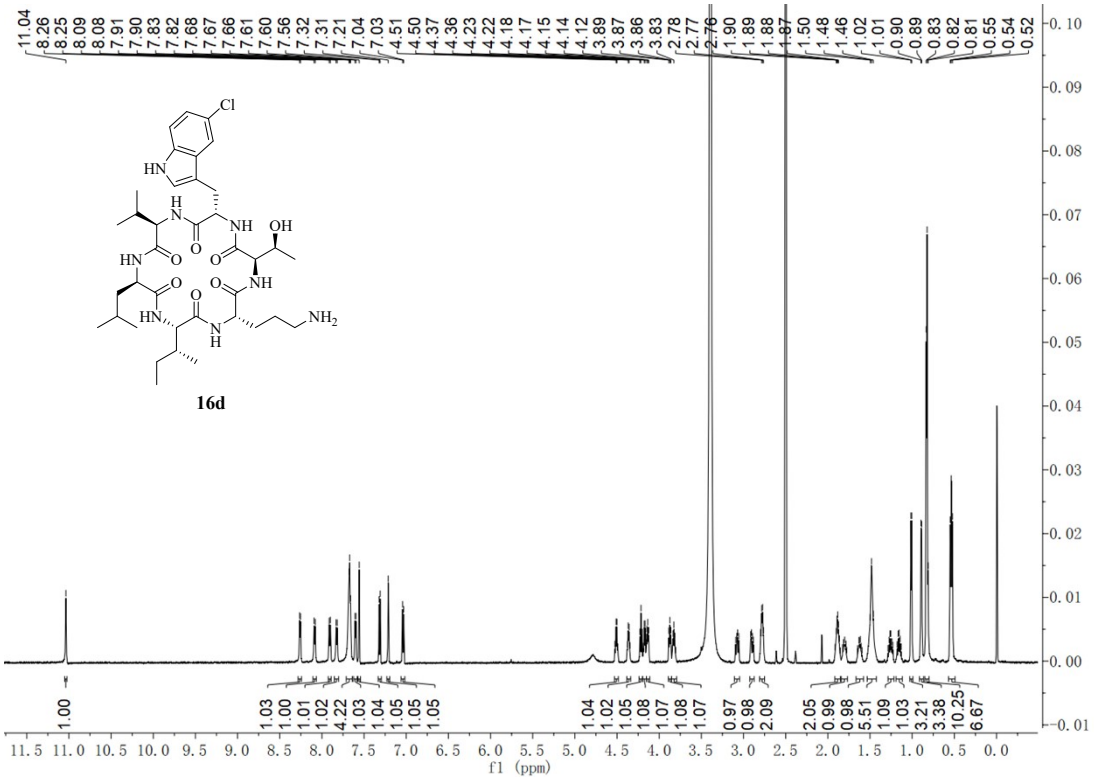


Figure S31. ^{1}H NMR spectrum of compound **16d**.

7、Refencences

1. Shin, Y.-H.; Bae, S.; Sim, J.; Hur, J.; Jo, S.-I.; Shin, J.; Suh, Y.-G.; Oh, K.-B.; Oh, D.-C., Nicrophorusamides A and B, antibacterial chlorinated cyclic peptides from a gut bacterium of the carrion beetle Nicrophorus concolor. *J. Nat. Prod.* **2017,** *80*, 2962-2968.