Supporting Information

Total synthesis of Nannocystin Ax

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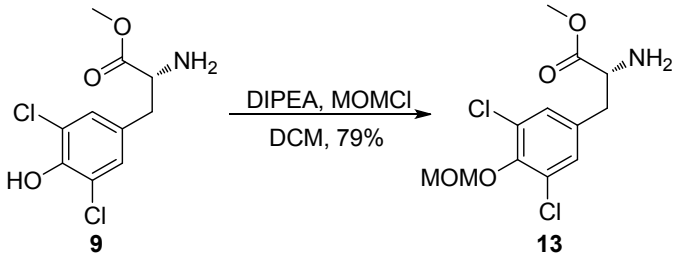
TABLE OF CONTENTS

1. General Information

All reactions were manipulated in flame-dried glassware under argon atmosphere using dried and deoxygenated solvents, unless stated otherwise. THF and Et_2O were distilled from sodium and benzophenone. CH_2Cl_2 and Et_3N were distilled from calcium hydride immediately prior to use. (4*S*)-3-[(1*E*)-1-(tert-Butyldimethylsilyoxy)-2-methylbuta-1, 3-dienyl]-4-isopropyloxazolidin-2-one,[1] (2*S*, 3*S*)-2-[(tert-butoxy carbonyl)-(methyl)amino]-3-methylpentanoic acid[2] and (2*R*)-2-*N*-(Boc)amino-3-methyl-1,3-butanediol[3] were prepared as known methods. Analytical thin layer chromatography was performed on precoated glass purchased and visualized by UV, potassium permanganate, *p*-anisaldehyde, CAM staining. ^1H and ^{13}C NMR spectra were recorded on Brukur (400 M and 100 M, respectively) and Brukur (600 M and 150 M, respectively) NMR spectrometer with TMS as the internal standard and were calibrated using residual undeuterated solvent as an internal reference ($CDCl_3$: 7.26 (^1H) and 77.16 ppm (^{13}C); $(CD_3)_2CO$: 2.05 (^1H) and 29.84, 206.26 ppm (^{13}C); DMSO: 2.50 (^1H) and 39.52 ppm (^{13}C)). The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Coupling constants (*J*) were reported in Hertz (Hz). High resolution mass spectra (HRMS) were obtained from Sichuan University MS facility using electron ionization mode or mixed ionization mode. Infrared (IR) spectra were recorded on a Spectrometer and reported in frequency of absorption. Optical rotations were measured on a Jasco-P-2000 polarimeter using a 100 mm length cell at 589 nm.

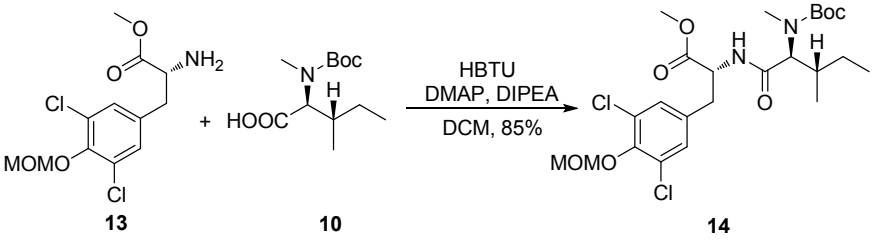
2. Experimental Procedures and Characterization

Synthesis of 13



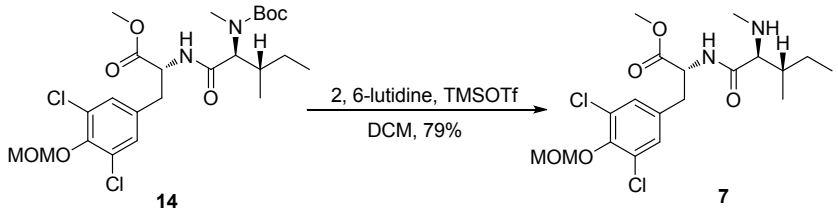
To a solution of **9** (1.61 g, 6.1 mmol) in CH_2Cl_2 (30 mL) and DIPEA (2.5 mL, 15.2 mmol) was added MOMCl (0.9 mL, 12.2 mmol) dropwise at 0 ℃. The mixture was allowed to warm to room temperature and stirred for additional 8 h until the reaction was completed. The reaction mixture was concentrated in vacuo. The crude was redissolved with EtOAc (50 mL), washed with 1 N HCl (20 mL). After the mixture was adjusted to pH 8 with saturated aqueous $NaHCO_3$ at 0 ℃, the aqueous layer was extracted with EtOAc (100 mL × 3). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. The crude residue was purified by column chromatography on silica gel (petroleum ether/EtOAc = 1/1), affording **13** (1.49 g, 79%) as a colorless oil. R_f = 0.25 (petroleum ether/EtOAc = 1/1); $[\alpha]^{21}_D$ = – 14.8 (*c* 0.9, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.14 (s, 2H), 5.13 (s, 2H), 3.70 (s, 3H), 3.65 (s, 4H), 2.96 (dd, *J* = 13.7, 5.0 Hz, 1H), 2.72 (dd, *J* = 13.7, 8.0 Hz, 1H), 1.54 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.0, 148.5, 135.4, 129.8, 129.3, 99.4, 58.2, 55.5, 52.2, 39.9; IR (KBr) ν_{max}: 3385, 2952, 2846, 1738, 1597, 1556, 1475, 1440, 1257, 1203, 1099, 937 cm^{-1}; HRMS–ESI(*m/z*): [M+Na]$^+$calculated for $C_{12}H_{15}C_{l2}NNaO_4^+$, 330.0276, found: 330.0280.

Synthesis of 14



To a solution of amine **13** (958.6 mg, 3.1 mmol) and acid **10** (772.5 mg, 3.1 mmol) in CH_2Cl_2 (30 mL), HBTU (3.49 g, 9.3 mmol), DMAP (76.0 mg, 0.62 mmol) and DIPEA (2.0 mL, 12.4 mmol) were added sequentially. The mixture was stirred at room temperature for 12 h and filtrated through celite. The filtrate was washed by 1 N HCl (20 mL) and saturated aqueous $NaHCO_3$ (20 mL) successively. The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue afforded amide **14** (1.42 g, 85%) as a colorless oil after purification by column chromatography on silica gel (petroleum ether/EtOAc = 5/1). R_f = 0.60 (petroleum ether/EtOAc = 3/1); $[\alpha]^{21}_D$ = – 61.6 (*c* 1.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.08 (s, 2H), 6.83 (d, *J* = 7.5 Hz, 1H), 5.12 (s, 2H), 4.77 (d, *J* = 5.5 Hz, 1H), 4.09 (d, *J* = 11.2 Hz, 1H), 3.69 (s, 3H), 3.65 (s, 3H), 3.07 (dd, *J* = 14.0, 5.1 Hz, 1H), 2.88 (dd, *J* = 13.8, 7.7 Hz, 1H), 2.70 (s, 3H), 1.41 (s, 9H), 1.41 – 1.27 (m, 2H), 1.02 – 0.93 (m, 1H), 0.85 (t, *J* = 7.3 Hz, 3H), 0.77 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.2, 170.6, 157.3, 148.7, 134.2, 129.8, 129.4, 99.4, 80.6, 62.9, 58.2, 52.7, 52.5, 36.9, 31.5, 30.3, 28.4, 24.5, 15.8, 10.6; IR (KBr) ν_{max}: 3345, 2968, 1847, 1747, 1684, 1516, 1476, 1313, 1256, 1206, 1159, 937, 801 cm^{-1}; HRMS–ESI (*m/z*): $[M+Na]^+$ calculated for $C_{24}H_{36}Cl_2N2NaO_7^+$, 557.1797, found: 557.1790.

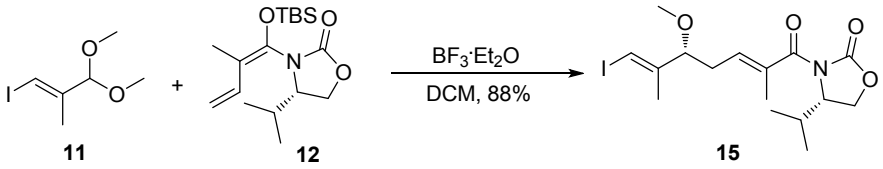
Synthesis of 7



To a solution of amide **14** (1.34 g, 2.5 mmol) in CH_2Cl_2 (25 mL) was added 2, 6-lutidine (1.7 mL, 14.9 mmol) and TMSOTf (1.8 mL, 9.9 mmol) sequentially at 0 ℃. The reaction was stirred for 1 h and quenched by adding saturated aqueous $NaHCO_3$ (50 mL). The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (100 mL × 4). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. After purification by column chromatography on silica gel (CH_2Cl_2/MeOH = 20/1), it furnished amine **7** (852.8 mg, 79%) as a colorless

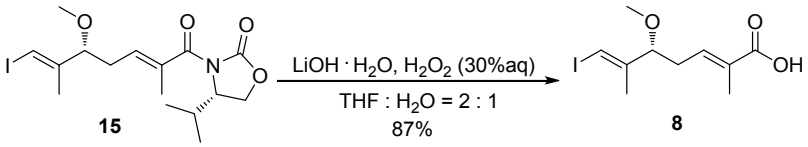
oil. R_f = 0.50 (CH_2Cl_2/MeOH = 20/1); $[\alpha]^{21}_D$ = – 28.2 (*c* 0.9, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.67 (d, *J* = 7.7 Hz, 1H), 7.09 (s, 2H), 5.12 (s, 2H), 4.80 (dd, *J* = 12.8, 6.1 Hz, 1H), 3.72 (s, 3H), 3.65 (s, 3H), 3.12 (dd, *J* = 14.0, 5.2 Hz, 1H), 2.98 (dd, *J* = 13.9, 7.0 Hz, 1H), 2.80 – 2.79 (m, 1H), 2.25 (s, 3H), 1.76 (s, 1H), 1.45 – 1.41 (m, 2H), 1.14 – 1.03 (m, 1H), 0.91 (d, *J* = 6.8 Hz, 3H), 0.85 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.6, 171.6, 148.7, 134.2, 129.9, 129.4, 99.4, 69.9, 58.2, 52.5, 52.3, 38.5, 36.8, 36.4, 25.0, 15.9, 11.9; IR (KBr) ν_{max} : 3309, 2961, 1745, 1653, 1511, 1475, 1256, 1212, 1161, 935, 780 cm^{-1}; HRMS–ESI (*m/z*): [M+Na]$^+$calculated for $C_{19}H_{28}C_{l2}NaO_5^+$, 457.1273; found: 457.1277.

Synthesis of 15



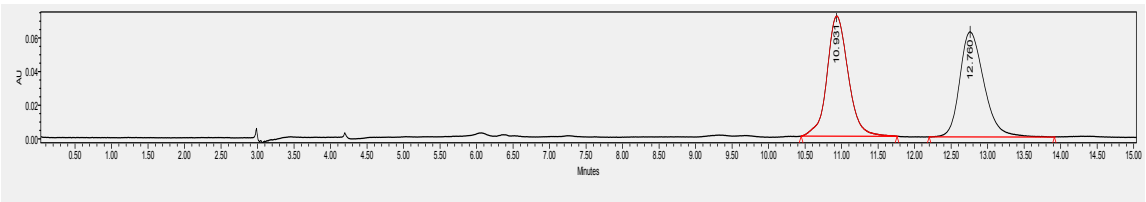
To a solution of acetal **11** (2.70 g, 11.2 mmol) in CH_2Cl_2 (28 mL) was added $BF_3{\cdot}Et_2O$ (1.38 mL, 11.2 mmol) at – 78 ℃. After stirring for 15 min, **12** (1.58 g, 11.2 mmol) in CH_2Cl_2 (28 mL) was added dropwise. After stirring for 4.5 h at – 60 ℃, the reaction was quenched by pyridine (3.6 mL, 44.6 mmol). The mixture was warmed to room temperature and saturated aqueous $NaHCO_3$ (56 mL) was added. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (100 mL × 4). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc = 5:1) to furnish **15** (4.13 g, 88%, 14:1 *dr*) as a yellowish solid. R_f = 0.45 (petroleum ether/EtOAc = 5:1); $[\alpha]^{24}_D$ = – 21.2 (*c* 0.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.25 (s, 1H), 5.97 (t, *J* = 7.2 Hz, 1H), 4.53 – 4.48 (m, 1H), 4.31 (t, *J* = 8.9 Hz, 1H), 4.17 (dd, *J* = 8.9, 5.3 Hz, 1H), 3.73 (t, *J* = 6.8 Hz, 1H), 3.20 (s, 3H), 2.52 – 2.44 (m, 1H), 2.39 – 2.31 (m, 2H), 1.89 (s, 3H), 1.77 (s, 3H), 0.90 (t, *J* = 7.5 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.7, 153.7, 147.2, 134.1, 132.8, 84.6, 80.0, 63.6, 58.3, 56.6, 32.8, 28.4, 18.8, 18.0, 15.2, 14.0; IR (KBr) ν_{max}: 3056, 2965, 2922, 2852, 1784, 1682, 1462, 1368, 1269, 1208, 1098, 739 cm^{-1}; HRMS–ESI(*m/z*): [M+Na]$^+$ calculated for $C_{16}H_{24}INNaO_4^+$, 444.0648, found: 444.0646.

Synthesis of Acid 8



To a solution of amide **15** (1.05 g, 2.5 mol) in THF (16 mL) and water (8 mL) was added LiOH·H_2O (314.7 mg, 7.5 mmol) and H_2O_2 (0.78 mL, 7.5 mmol, 30% in H_2O). After stirring at room temperature for 24 h, the reaction was quenched by adding 2 N HCl (15 mL). The solution was extracted with EtOAc (50 mL × 3), the combined organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. It furnished **8** (674.5 mg, 87%, 89% *ee*) as a yellowish oil after purification by column chromatography on silica gel (petroleum ether/EtOAc = 2:1). The ee value was determined by SFC; SFC (Chiral ND 5u, CO_2/MeOH = 95/5, flow rate 1.0 mL/min, λ = 254 nm); R_f = 0.55 (petroleum ether/EtOAc = 2/1); $[\alpha]^{24}_D$ = – 28.4 (*c* 0.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.83 (t, *J* = 7.1 Hz, 1H), 6.27 (s, 1H), 3.76 (dd, *J* = 7.3, 6.1 Hz, 1H), 3.21 (s, 3H), 2.55 – 2.47 (m, 1H), 2.40 – 2.35 (m, 1H), 1.82 (s, 3H), 1.77 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.3, 147.1, 140.1, 129.1, 84.8, 80.0, 56.7, 33.7, 18.9, 12.4; IR (KBr) ν_{max}: 2927, 1687, 1645, 1422, 1280, 1099 cm^{-1}; HRMS–ESI(*m/z*): $[M+Na]^+$calculated for $C_{10}H_{15}INaO_3^+$, 332.9964, found: 332.9965.

Racemic 8



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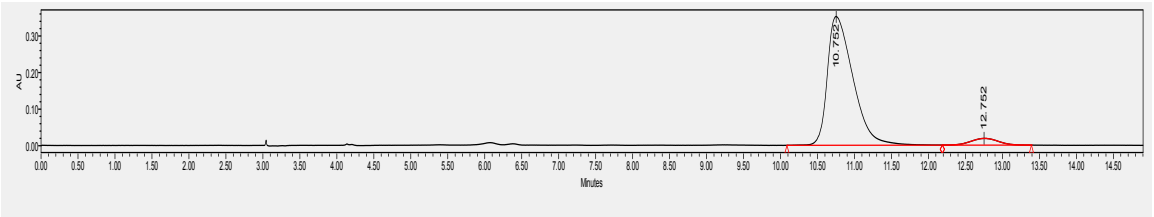
2	12.760	1434369	49.96	62493	bb	Unknown

Chiral 8



	Retention Time	Area	% Area	Height	Int Type	Peak Type
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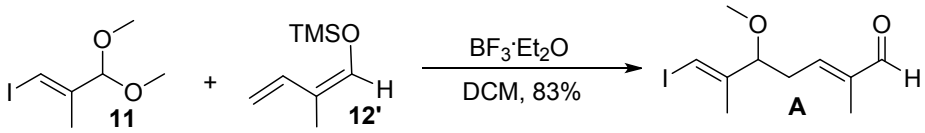
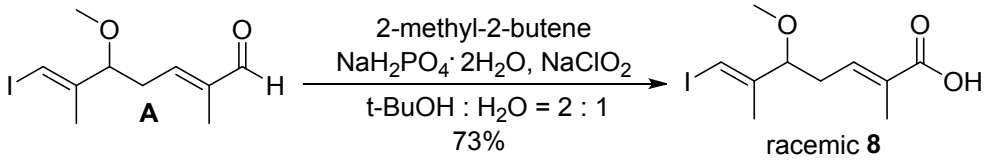
Synthesis of *rac*-8

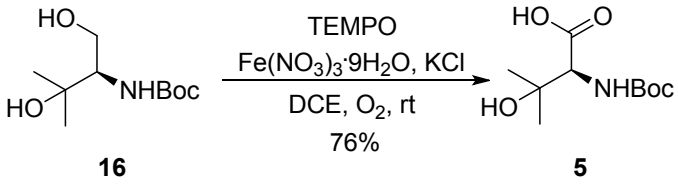


To a solution of acetal **11** (242.0 mg, 1.0 mmol) in CH_2Cl_2 (2.5 mL) was added $BF_3{\cdot}Et_2O$ (0.15 mL, 1.0 mmol, $BF_3{\cdot}Et_2O$ was freshly distilled before use) at –78 °C. After stirring for 15 min, the silyl dienolether **12′** (0.19 mL, 1.0 mmol) in CH_2Cl_2 (3 mL) was dropwise added.[4] After stirring for 8 h at –60 °C, the reaction was quenched by pyridine (0.34 mL). The mixture was warmed to room temperature and saturated $NaHCO_3$ (10 mL) was added. The organic were separated and the aqueous layer was extracted with EtOAc (20 mL x 5). The combined organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue purified by column chromatography on silica gel (petroleum ether /EtOAc = 30:1 eluent) to furnish the aldehyde **A** (348.0 mg, 83%) as yellowish oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.38 (s, 1H), 6.45 – 6.41 (m, 1H), 6.28 (s, 1H), 3.79 (dd, J = 7.8, 5.7 Hz, 1H), 3.21 (s, 3H), 2.66 – 2.59 (m, 1H), 2.54 – 2.47 (m, 1H), 1.77 (d, J = 1.1 Hz, 3H), 1.72 (d, J = 1.0 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 195.0, 149.2, 146.9, 140.9, 84.5, 80.0, 56.7, 33.6, 19.0, 9.5. IR (KBr) ν_{max}: 2928, 1687, 1646, 1441, 1260, 1100 cm^{-1}; LRMS–ESI(*m/z*): $[M+Na]^+$calculated for $C_{10}H_{15}INaO_2^+$, 317.00, found: 317.07.



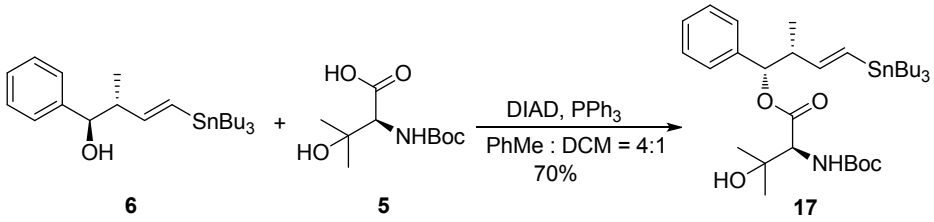
2-methyl-2-butene (0.2 mL, 1.7 mmol), $NaH_2PO_4 \cdot 2H_2O$ (106.1 mg, 0.68 mmol) and $NaClO_2$ (62.1 mg, 0.68 mmol) were sequentially added to a solution of the aldehyde **A** (50.0 mg, 0.17 mmol) in *t*-BuOH/H_2O (2:1 v/v, 3 mL). After stirring at room temperature for 4 h, diluted with brine (10 mL) and the aqueous layer was extracted with EtOAc (10 mL × 3). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. After purification by column chromatography on silica gel (petroleum ether/EtOAc = 2/1), it furnished racemic **8** (38.5 mg, 73%) as a yellowish oil. R_f = 0.55 (petroleum ether/EtOAc = 2/1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.83 (t, J = 7.1 Hz, 1H), 6.27 (s, 1H), 3.76 (dd, J = 7.3, 6.1 Hz, 1H), 3.21 (s, 3H), 2.55 – 2.47 (m, 1H), 2.40 – 2.35 (m, 1H), 1.82 (s, 3H), 1.77 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.3, 147.1, 140.1, 129.1, 84.8, 80.0, 56.7, 33.7, 18.9, 12.4; IR (KBr) ν_{max}: 2927, 1687, 1645, 1422, 1280, 1099 cm^{-1}; LRMS–ESI(*m/z*): $[M–H]^-$calculated for $C_{10}H_{14}IO_3^-$, 309.00, found: 309.01.

Synthesis of 5



TEMPO (156.3 mg, 1.0 mmol), $Fe(NO_3)_3 \cdot 9H_2O$ (404.0 mg, 1.0 mmol) and KCl (74.6 mg, 1.0 mmol) were added a stirred solution of (2*R*)-2-*N*-(Boc)amino-3-methyl-1,3-butanediol **16** (2.19 g, 10.0 mmol) under O_2 atmosphere in dry DCE (100 mL). The reaction was then stirred at room temperature until completion of the reaction as monitored by TLC (48 h). The reaction mixture was concentrated in vacuo and the residue was purified by column chromatography (CH_2Cl_2/MeOH = 20/1), affording **5** (1.80 g, 76%) as a yellowish solid. The spectroscopic data are consistent with those reported in literature.[3]

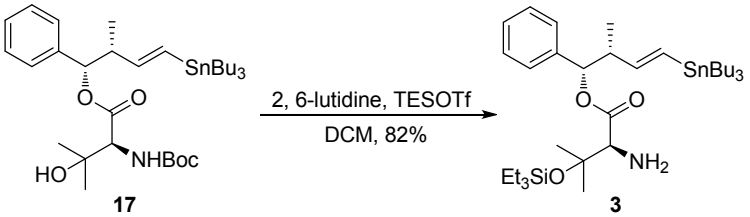
Synthesis of 17



6 5 17

PPh_3 (1.85 g, 7.1 mmol) and acid **5** (510.7 mg, 2.1 mmol) was weighted into flask in glove bag. The flask was capped with rubber spetum and removed from glove box and placed in cold bath. Alcohol **6** (645.4 mg, 1.4 mmol) dissolved in toluene (12 mL) was added and the mixture was maintained to 0 ℃. DIAD (0.85 mL, 4.3 mmol) was added dropewise. After 30 min, the mixture was added CH_2Cl_2 (3 mL) and allowed to warm to room temperature and stirred 4 h. The reaction was quenched by saturated aqueous $NaHCO_3$ (10 mL), the aqueous layer was extracted with EtOAc (40 mL × 4). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on deactivated silica gel (petroleum ether/EtOAc = 10/1), affording **17** (668.0 mg, 70%, 10:1 *dr*) as a colorless oil. R_f = 0.30 (petroleum ether/EtOAc = 10/1); $[\alpha]^{24}_D$ = – 41.5 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.28 – 7.24 (m, 5H), 5.86 (d, *J* = 19.0 Hz, 1H), 5.70 – 5.63 (m, 1H), 5.62 (d, *J* = 8.2 Hz, 1H), 5.39 (d, *J* = 8.9 Hz, 1H), 4.22 (d, *J* = 9.1 Hz, 1H), 2.80 – 2.75 (m, 1H), 2.63 (s, 1H), 1.44 (s, 9H), 1.41 – 1.34 (m, 6H), 1.29 – 1.21 (m, 6H), 1.18 (s, 3H), 1.12 (d, *J* = 6.7 Hz, 3H), 1.06 (s, 3H), 0.88 – 0.84 (m, 9H), 0.80 – 0.76 (m, 6H) ; ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.6, 155.9, 148.5, 138.5, 130.2, 128.2, 127.6, 81.1, 80.2, 72.0, 61.3, 46.5, 29.1, 28.4, 27.4, 27.1, 26.9, 26.4, 16.1, 13.9, 9.5 ; IR (KBr) ν_{max}: 3440, 2959, 2926, 1720, 1496, 1370, 1163, 1052, 989, 698 cm^{-1}; HRMS–ESI (*m/z*): $[M+Na]^+$ calculated for $C_{33}H_{57}NNaO_5Sn^+$, 690.3156; found 690.3161.

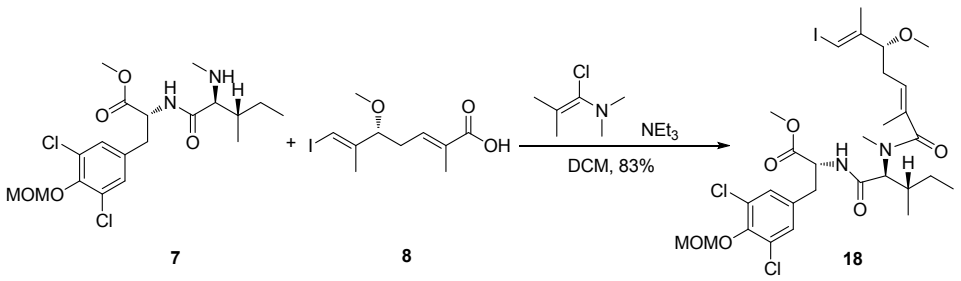
Synthesis of 3



17 3

To a solution of **17** (665.6 mg, 1.0 mmol) in CH_2Cl_2 (10 mL), 2, 6-lutidine (0.7 mL, 6.0 mmol) and TESOTf (0.9 mL, 4.0 mmol) were added at 0 ℃. After stirring at 0℃ for 30 min, saturated aqueous $NaHCO_3$ (10 mL) was added to quench the reaction. After separation, the aqueous layer was extracted with CH_2Cl_2 (50 mL × 3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography on deactivated silca gel (petroleum ether/EtOAc = 30/1) to furnish **3** (558.7 mg, 82%, 14:1 *dr*) as a colorless oil. R_f = 0.52 (petroleum ether/EtOAc = 10/1); $[\alpha]^{22}_D$ = + 5.1 (*c* 1.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.27 – 7.22 (m, 5H), 5.83 (d, *J* = 19.1 Hz, 1H), 5.67 (dd, *J* = 19.1, 6.8 Hz, 1H), 5.61 (d, *J* = 7.9 Hz, 1H), 3.37 (s, 1H), 2.76 (dd, *J* = 14.3, 6.9 Hz, 1H), 1.73 (s, 2H), 1.40 – 1.34 (m, 6H), 1.25 (s, 3H), 1.30 – 1.19 (m, 6H), 1.08 (d, *J* = 6.1 Hz, 3H), 1.07 (s, 3H), 0.91 – 0.81 (m, 18H), 0.78 – 0.74 (m, 6H), 0.52 (q, *J* = 7.9 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 172.7, 148.7, 138.7, 129.8, 128.0, 127.9, 127.9, 80.2, 75.1, 65.2, 46.3, 29.1, 28.0, 27.3, 25.5, 16.3, 13.8, 9.4, 7.1, 6.7; ^{1}H NMR (400 MHz, C_6D_6): δ 7.32 (d, *J* = 7.8 Hz, 2H), 7.18 – 7.14 (m, 2H), 7.06 (t, *J* = 7.4 Hz, 1H), 6.02 (d, *J* = 19.0 Hz, 1H), 5.94 – 5.89 (m, 2H), 3.44 (s, 1H), 2.89 – 2.80 (m, 1H), 1.59 – 1.49 (m, 6H), 1.37 – 1.32 (m, 6H), 1.31 (s, 3H), 1.19 (s, 3H), 1.15 (d, *J* = 6.7 Hz, 3H), 0.97 – 0.88 (m, 24H), 0.51 (q, *J* = 7.9 Hz, 6H). ^{13}C NMR (101 MHz, C_6D_6): δ 172.9, 149.7, 139.5, 129.6, 128.2, 127.9, 79.7, 75.7, 65.4, 47.0, 29.5, 27.7, 27.6, 26.3, 16.3, 14.0, 9.7, 7.3, 7.0. IR (KBr) ν_{max}: 3400, 2957, 2926, 2876, 1737, 1598, 1459, 1377, 1239, 1148, 1040, 744 cm^{-1}; HRMS–ESI (*m/z*): $[M+Na]^+$ calculated for $C_{34}H_{64}NO_3SiSn^+$, 682.3677; found: 682.3677.

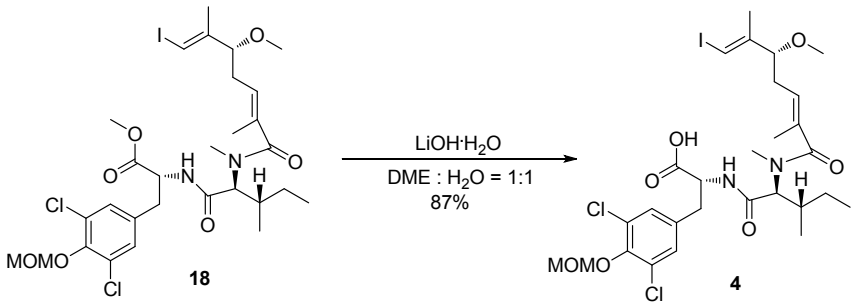
Synthesis of 18



To a solution of acid **8** (632.2 mg, 2.0 mmol) in CH_2Cl_2 (20 mL), 1-chloro-N, N, 2-trimethylprophenylamine (0.35 mL, 2.4 mmol) was added dropwise. After stirring at

room temperature for 2 h, the amine **7** (980.2 mg, 2.2 mmol) and Et_3N (0.85 mL, 6.1 mmol) were added in succession and stirred overnight. The mixture was quenched by saturated aqueous $NaHCO_3$ (10 mL). After separation, the aqueous layer was extracted with CH_2Cl_2 (50 mL × 4). The combined organic layers were dried over Na_2SO_4, filtrated and concentrated under reduced pressure. The residue was purified by column chromatography on silca gel (petroleum ether/EtOAc = 3/1), furnishing **18** (1.23 g, 83%) as a colorless oil. R_f = 0.48 (petroleum ether/EtOAc = 3/1); $[\alpha]^{24}_D$ = – 85.4 (*c* 0.6, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.12 (s, 2H), 7.03 (d, *J* = 8.4 Hz, 1H), 6.23 (s, 1H), 5.45 (t, *J* = 6.8 Hz, 1H), 5.13 (s, 3H), 4.77 – 4.71 (m, 1H), 4.48 (d, *J* = 11.5 Hz, 1H), 3.74 – 3.73 (m, 1H), 3.70 (s, 3H), 3.66 (s, 3H), 3.18 (s, 3H), 3.10 (dd, *J* = 14.2, 5.0 Hz, 1H), 2.86 (dd, *J* = 14.2, 8.6 Hz, 1H), 2.80 (s, 3H), 2.44 – 2.37 (m, 1H), 2.36 – 2.28 (m, 1H), 2.13 – 2.07 (m, 1H), 1.79 (s, 3H), 1.75 (s, 3H), 1.37 – 1.29 (m, 1H), 1.04 – 0.95 (m, 1H), 0.87 (t, *J* = 7.3 Hz, 3H), 0.82 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 175.3, 171.3, 170.1, 148.7, 147.4, 134.3, 133.5, 129.7, 129.4, 127.6, 99.4, 85.1, 79.7, 60.9, 58.2, 56.6, 52.7, 52.6, 36.7, 33.1, 32.1, 30.9, 24.6, 18.8, 15.7, 14.3, 10.5; IR (KBr) ν_{max}: 3316, 2962, 2930, 1745, 1682, 1613, 1527, 1474, 1257, 1162, 1100, 938, 800 cm^{-1}; HRMS–ESI (*m/z*): $[M+Na]^+$ calculated for $C_{29}H_{41}Cl_2IN_2NaO_7^+$, 749.1233; found: 749.1224.

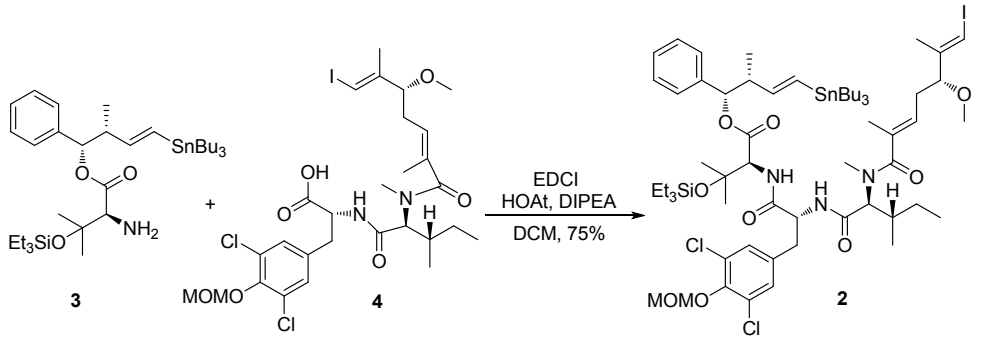
Synthesis of 4



To a solution of **18** (792.7 mg, 1.1 mmol) in 1, 2-dimethoxyethane (10 mL), $LiOH·H_2O$ (230.1 mg, 5.5 mmol) in water (10 mL) was added. After stirring at room temperature for 8 h, the mixture was adjusted to pH 2 with 1 N HCl at 0 ℃, then extracted with CH_2Cl_2 (50 mL × 4). The combined organic layers were dried over Na_2SO_4, filtrated and concentrated under reduced pressure. The crude product was

purified by column chromatography on silica gel (CH_2Cl_2/MeOH = 25/1), furnishing **4** (676.5 mg, 87%) as a colorless oil. R_f = 0.4 (CH_2Cl_2/MeOH = 20/1); $[\alpha]^{22}_D$ = – 56.1 (*c* 1.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (s, 1H), 7.28 (d, *J* = 7.8 Hz, 1H), 7.17 (s, 2H), 6.21 (s, 1H), 5.40 (t, *J* = 6.6 Hz, 1H), 5.10 (s, 2H), 4.74 (d, *J* = 5.3 Hz, 1H), 4.52 (d, *J* = 11.2 Hz, 1H), 3.70 (t, *J* = 6.7 Hz, 1H), 3.65 (s, 3H), 3.15 (s, 3H), 3.15 – 3.10 (m, 1H), 2.92 – 2.89 (m, 1H), 2.89 (s, 3H), 2.35 (dd, *J* = 14.1, 7.1 Hz, 1H), 2.27 (dd, *J* = 14.6, 7.3 Hz, 1H), 2.07 – 2.00 (m, 1H), 1.72 (s, 3H), 1.71 (s, 3H), 1.34 – 1.25 (m, 1H), 1.02 – 0.93 (m, 1H), 0.87 (t, *J* = 7.2 Hz, 3H), 0.76 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 175.4, 173.3, 169.5, 148.5, 147.3, 135.0, 133.0, 130.1, 129.2, 128.1, 99.4, 85.0, 79.9, 61.4, 58.3, 56.6, 52.9, 36.6, 33.4, 32.0, 31.5, 24.8, 18.9, 15.6, 14.2, 10.6; IR (KBr) ν_{max}: 3330, 2965, 2930, 1737, 1681, 1610, 1475, 1402, 1257, 1162, 1099, 939, 800 cm^{-1}; HRM–ESI (*m/z*): $[M+Na]^+$ calculated for $C_{28}H_{39}Cl_2IN_2NaO_7^+$,735.1077; found: 735.1082.

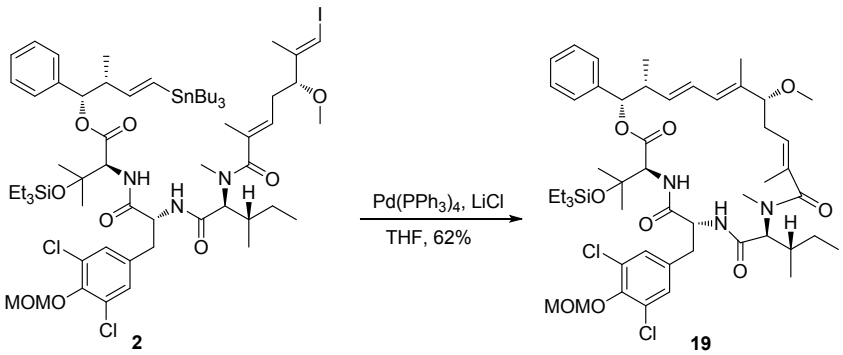
Synthesis of 2



To a solution EDCI (172.6 mg, 0.90 mmol), HOAt (127.1 mg, 0.90 mmol), and DIPEA (0.2 mL, 1.2 mmol) in CH_2Cl_2 (4 mL) was added acid **4** (251.0 mg, 0.35 mmol) and amine **3** (202.1 mg, 0.30 mmol) in CH_2Cl_2 (2 mL) at 0 ℃. After being stirred for 1 h, the reaction mixture was allowed to warm to room temperature and stirred for additional 7 h. The reaction mixture was concentrated in vacuo and the residue was purified by column chromatography (petroleum ether/EtOAc = 5/1), affording **2** (306.4 mg, 75%) as a colorless oil. R_f = 0.35 (petroleum ether/EtOAc = 5/1); $[\alpha]^{22}_D$ = – 40.1 (*c* 1.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.30 – 7.21 (m, 6H), 7.21 (s, 2H), 7.10 (d, *J* = 8.1 Hz, 1H), 6.72 (d, *J* = 8.5 Hz, 1H), 6.27 (s, 1H), 5.81 (d, *J* = 19.0 Hz, 1H), 5.60 (dd, *J* = 19.0, 6.8 Hz, 1H), 5.51 – 5.44 (m, 2H), 5.14 (s,

2H), 4.64 (q, J = 7.5 Hz, 1H), 4.49 (d, J = 11.3 Hz, 1H), 4.33 (d, J = 8.5 Hz, 1H), 3.78 (t, J = 6.8 Hz, 1H), 3.69 (s, 3H), 3.22 (s, 3H), 3.16 – 3.11 (m, 1H), 2.93 – 2.86 (m, 1H), 2.84 (s, 3H), 2.83 – 2.77 (m, 1H), 2.46 (dd, J = 14.5, 6.5 Hz, 1H), 2.36 (dd, J = 14.7, 7.3 Hz, 1H), 2.15 – 2.13 (m, 2H),1.86 (s, 3H), 1.78 (s, 3H), 1.44 – 1.34 (m, 7H), 1.29 – 1.20 (m, 7H), 1.12 (d, J = 5.6 Hz, 3H), 1.11 (s, 3H), 1.04 (s, 3H), 0.93 – 0.86 (m, 13H), 0.84 – 0.75 (m, 18H), 0.40 (q, J = 7.7 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 175.2, 170.5, 170.0, 169.3, 148.4, 147.5, 138.5, 135.1, 133.6, 130.2, 129.9, 129.5, 128.3, 128.1, 128.0, 127.4, 99.4, 85.2, 81.1, 79.8, 74.4, 61.6, 58.3, 56.6, 54.1, 46.4, 36.6, 33.6, 32.1, 31.4, 29.2, 29.1, 29.0, 28.4, 27.7, 27.4, 24.7, 18.8, 16.9, 15.8, 14.4, 13.9, 10.6, 9.5, 7.1, 6.5; IR (KBr) ν_{max}: 3319, 2958, 2927, 1739, 1688, 1608, 1518, 1460, 1374, 1257, 1161, 947 cm^{-1}; HRMS–ESI (m/z): $[M+Na]^+$ calculated for $C_{62}H_{100}Cl_2IN_3NaO_9SiSn^+$, 1398.4570; found 1398.4562.

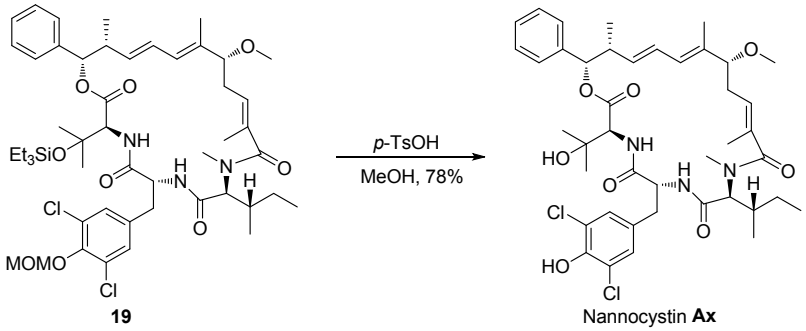
Synthesis of 19



$Pd(PPh_3)_4$ (15.7 mg, 0.01 mmol) and LiCl (26.8 mg, 0.60 mmol) was weighted into flask in glove bag. The flask was capped with rubber spetum and removed from glove box. Compound **2** (272.8 mg, 0.20 mmol) dissolved in THF (100 mL) was added and the mixture was stirred at 60 ℃ for 18 h. Until the mixture was cold to room temperature, water (20 mL) was added to quench the reaction. The aqueous layer was extracted with EtOAc (50 mL × 3). The combined organic layers were dried over Na_2SO_4, filtrated and concentrated in vacuo. The crude product was purified by column chromatography on silica gel (petroleum ether/EtOAc = 3/1), furnishing **19** (118.7 mg, 62 %) as a colorless oil. R_f = 0.33 (petroleum ether /EtOAc = 3/1); $[\alpha]^{22}_D$ = – 40.3 (c 0.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32 – 7.22 (m, 5H), 7.19 (s,

2H), 6.96 (d, J = 8.9 Hz, 1H), 6.76 (d, J = 8.8 Hz, 1H), 6.30 (dd, J = 15.1, 10.7 Hz, 1H), 5.93 (s, 1H), 5.85 (d, J = 10.6 Hz, 1H), 5.74 (dd, J = 15.3, 5.4 Hz, 1H), 5.42 – 5.39 (m, 1H), 5.11 (s, 2H), 4.62 (d, J = 11.5 Hz, 1H), 4.52 – 4.47 (m, 1H), 4.40 (d, J = 8.8 Hz, 1H), 3.65 (s, 3H), 3.60 (dd, J = 10.6, 3.0 Hz, 1H), 3.21 (s, 3H), 3.06 (dd, J = 13.1, 10.3 Hz, 1H), 2.75 (dd, J = 13.2, 5.3 Hz, 1H), 2.61 (s, 3H), 2.61 – 2.55 (m, 1H), 2.39 – 2.36 (m, 1H), 2.19 – 2.13 (m, 1H), 1.82 (s, 3H), 1.76 (s, 3H), 1.35 (s, 3H), 1.27 – 1.20 (m, 1H), 1.09 (d, J = 6.8 Hz, 3H), 1.05 – 0.98 (m, 1H), 0.92 – 0.87 (m, 7H), 0.83 (s, 3H), 0.76 (t, J = 7.9 Hz, 9H), 0.37 (q, J = 7.9 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 176.0, 170.6, 169.7, 169.5, 148.7, 139.2, 136.8, 135.5, 134.6, 133.6, 130.3, 129.5, 128.6, 128.2, 127.7, 127.7, 126.8, 125.6, 99.4, 85.8, 80.0, 75.7, 61.00, 60.1, 58.3, 56.1, 54.9, 42.2, 36.1, 32.4, 31.9, 30.5, 28.5, 27.4, 24.6, 16.1, 14.6, 11.6, 10.7, 10.5, 7.1, 6.6; IR (KBr) ν_{max}: 3424, 2925, 2854, 1739, 1619, 1459, 1381, 1258, 1157, 1069, 943, 800 cm^{-1}; HRMS–ESI (m/z): $[M+Na]^+$ calculated for $C_{50}H_{73}Cl_2N_3NaO_9Si^+$, 980.4391; found: 980.4393.

Synthesis of nannocystin Ax (1)



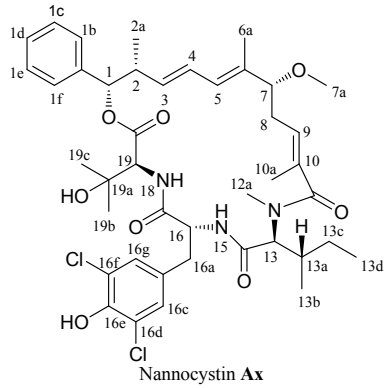
To a solution of **19** (116.7 mg, 0.12 mmol) in methanol (6 mL), *p*–TsOH (13.2 mg, 0.06 mmol) was added. The reaction mixture was stirred at room temperature for 4 h. After quenching by saturated aqueous $NaHCO_3$ (5 mL), the aqueous layer was extracted with EtOAc (30 mL × 3). The combined organic layers were dried over anhydrous $MgSO_4$, filtrated and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc = 2/1), furnishing nannocystin **Ax** (74.8 mg, 78%) as a white solid. And it was recrystallized from methanol to give nice crystalline needles nannocystin Ax (**1**). m.p. 177-178 °C; R_f = 0.45 (petroleum ether/EtOAc = 1/1); $[\alpha]^{23}_D$= – 67.4 (*c* 0.3, MeOH); ^{1}H NMR (600 MHz, DMSO): δ 9.81 (s, 1H), 8.52 (d, J = 9.9 Hz, 1H), 7.90 (d, J = 9.5 Hz, 1H),

7.54 (d, *J* = 7.6 Hz, 2H), 7.38 (s, 2H), 7.32 (t, *J* = 7.6 Hz, 2H), 7.25 (t, *J* = 7.3 Hz, 1H), 6.35 (m, 1H), 6.05 (m, 1H), 6.00 (m, 1H), 5.89 (br, 1H), 5.16 – 5.12 (m, 1H), 5.11 (s, 1H), 4.72 (m, 1H), 4.63 (m, 1H), 4.52 (d, *J* = 11.2 Hz, 1H), 3.53 (m, 1H), 3.08 (s, 3H), 2.80 (m, 1H), 2.74 (s, 3H), 2.65 (m, 1H), 2.59 (m, 1H), 2.35 (m, 2H), 1.73 (m, 1H), 1.72 (s, 3H), 1.65 (s, 3H), 1.22 (m, 1H), 1.11 (s, 3H), 1.02 (s, 3H), 0.93 (d, *J* = 6.8 Hz, 3H), 0.88 (m, 1H), 0.76 (t, *J* = 7.3 Hz, 3H), 0.42 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (150 MHz, DMSO): *δ* 172.8, 170.7, 170.5, 169.1, 147.3, 139.7, 137.3, 133.9, 133.5, 130.9, 129.6, 128.5, 127.8, 127.0, 126.1, 125.1, 124.8, 121.6, 84.9, 78.9, 71.7, 59.3, 58.9, 55.0, 52.9, 41.7, 36.5, 31.7, 31.1, 30.4, 28.1, 24.5, 24.0, 14.8, 14.4, 11.1, 10.2, 10.1; ^{13}C NMR (100 MHz, DMSO): *δ* 172.8, 170.7, 170.5, 169.1, 147.3, 139.8, 137.4, 133.9, 133.5, 130.9, 129.6, 128.5, 127.8, 127.0, 126.1, 125.1, 124.8, 121.6, 84.9, 78.9, 71.7, 59.3, 58.9, 55.0, 52.9, 41.7, 36.6, 31.7, 31.2, 30.5, 28.2, 24.5, 24.0, 14.8, 14.4, 11.1, 10.2, 10.0; ^{1}H NMR (400 MHz, Acetone): *δ* 8.67 (s, 1H), 7.63 (d, *J* = 8.9 Hz, 1H), 7.53 (d, *J* = 7.4 Hz, 2H), 7.32 (t, *J* = 7.2 Hz, 2H), 7.31 (s, 2H), 7.25 (t, *J* = 7.3 Hz, 1H), 7.12 (d, *J* = 9.0 Hz, 1H), 6.40 (dd, *J* = 14.6, 11.5 Hz, 1H), 5.99 (d, *J* = 10.8 Hz, 1H), 5.90 – 5.85 (m, 1H), 5.87 (d, *J* = 1.5 Hz, 1H), 5.46 (t, *J* = 7.1 Hz, 1H), 4.82 (dd, *J* = 15.3, 8.6 Hz, 1H), 4.68 (d, *J* = 9.5 Hz, 1H), 4.54 (d, *J* = 11.2 Hz, 1H), 4.18 (d, *J* = 1.5 Hz, 1H), 3.64 (dd, *J* = 10.0, 2.8 Hz, 1H), 3.16 (s, 3H), 3.07 – 3.02 (m, 1H), 2.84 – 2.77 (m, 1H), 2.75 – 2.70 (m, 1H), 2.72 (s, 3H), 2.56 – 2.46 (m, 1H), 2.39 – 2.36 (m, 1H), 2.01 – 1.94 (m, 1H), 1.81 (s, 3H), 1.73 (s, 3H), 1.42 – 1.35 (m, 1H), 1.20 (s, 3H), 1.05 (s, 3H), 0.99 (d, *J* = 6.9 Hz, 3H), 1.02 – 0.91 (m, 1H), 0.85 (d, *J* = 7.3 Hz, 3H), 0.75 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, Acetone): *δ* 175.6, 171.3, 171.1, 170.8, 148.6, 140.6, 137.2, 135.5, 134.5, 131.6, 130.4, 129.1, 128.7, 128.0, 127.6, 127.4, 126.6, 122.3, 86.1, 80.34, 72.9, 60.8, 60.7, 55.9, 54.4, 42.9, 37.4, 32.8, 32.1, 31.7, 28.6, 25.6, 25.2, 16.0, 14.6, 11.8, 11.2, 10.6; ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 7.35 – 7.25 (m, 6H), 7.02 (s, 2H), 6.52 (d, *J* = 7.7 Hz, 1H), 6.30 (dd, *J* = 15.0, 10.9 Hz, 1H), 5.87 (s, 1H), 5.85 (d, *J* = 9.1 Hz, 1H), 5.57 (dd, *J* = 15.1, 6.7 Hz, 1H), 5.45 (t, *J* = 7.3 Hz, 1H), 4.65 (dd, *J* = 15.1, 7.4 Hz, 1H), 4.59 (d, *J* = 8.9 Hz, 1H), 4.35 (d, *J* = 11.2 Hz, 1H), 3.64 (dd, *J* = 7.5, 2.3 Hz, 1H), 3.21 (s, 3H), 2.95 (dd, *J* = 13.7, 7.1 Hz, 1H), 2.81 (s, 3H), 2.73 (dd, *J* = 13.8, 6.5 Hz, 1H), 2.75 – 2.70 (m, 1H), 2.53 – 2.46 (m, 1H), 2.43 – 2.35 (m, 1H), 2.09 – 2.04 (m, 1H), 1.79 (s, 3H), 1.69 (s, 3H), 1.44 – 1.40 (m, 1H), 1.28 – 1.21 (m, 1H), 1.18 (s, 3H), 1.04 (s, 3H), 1.03 (d, *J* = 5.2 Hz, 3H), 0.92 (t, *J* = 7.2 Hz, 3H), 0.83 (d, *J* = 6.3 Hz, 3H); ^{13}C NMR (101 MHz,

$CDCl_3$): δ 176.2, 170.9, 170.3, 169.5, 146.9, 137.9, 134.9, 134.4, 133.3, 129.7, 129.1, 128.1, 128.1, 127.9, 127.2, 127.1, 126.7, 121.2, 85.0, 80.5, 72.3, 61.8, 60.8, 56.3, 53.9, 41.8, 36.1, 32.7, 31.5, 31.3, 27.0, 26.7, 25.4, 15.9, 14.4, 12.8, 12.8, 10.8; IR (KBr) ν_{max}: 3351, 2927, 2850, 1736, 1665, 1606, 1490, 1375, 1312, 1157, 1065, 970 cm^{-1}; HRMS–ESI (*m/z*): $[M+Na]^+$ calculated for $C_{42}H_{55}Cl_2N_3NaO_8^+$, 822.3264; found: 822.3260.

3. NMR comparison between synthetic and natural sample

Tabulated comparison of ^{1}H NMR data of the synthetic nannocystin Ax (**1**) with that reported by Dr. Dominic Hoepfner and coworkers (*Angew. Chem. Int. Ed.* 2015, *54*, 10149; *Angew.Chem.* 2015, *127*, 10287.)

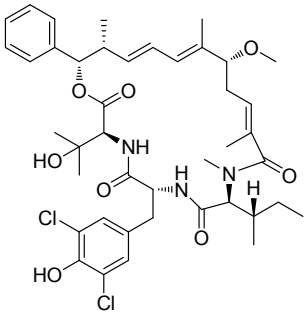


Nannocystin **Ax**

Hydrogen NO.	Nannocystin Ax (synthetic sample) δ_2, mult (J_2 in Hz)	Nannocystin Ax (synthetic sample) δ_1, mult (J_1 in Hz)	$\Delta_\delta=\delta_2-\delta_1$ ($\Delta_J=J_2-J_1$)
1	5.89 br	5.89 br	0
1b	7.54 d (7.6)	7.54 d (7.6)	0 (0)
1c	7.32 t (7.6)	7.32 t (7.6)	0 (0)
1d	7.25 d (7.6)	7.25 t (7.3)	0 (-0.3)
1e	7.32 t (7.6)	7.32 t (7.6)	0 (0)
1f	7.54 d (7.6)	7.54 d (7.6)	0 (0)
2	2.65 m	2.65 m	0
2a	0.93 d (6.7)	0.93 d (6.8)	0 (+0.1)
3	6.01 m	6.00 m	-0.01
4	6.35 m	6.35 m	0
5	6.02 m	6.05 m	+0.03
6a	1.65 s	1.65 s	0
7	3.53 m	3.54 m	+0.01
7a	3.07 s	3.08 s	+0.01
8	2.36 m	2.35 m	-0.01
9	5.13m	5.14m	+0.01
10a	1.71 s	1.72 s	+0.01

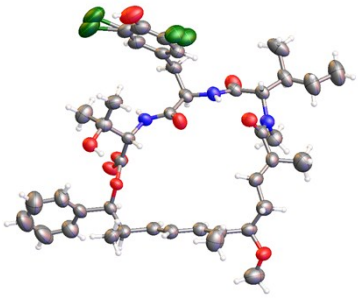
12a	2.74 s	2.74 s	0
13	4.51 d (11.1)	4.52 d (11.2)	+0.01 (+0.1)
13a	1.74 m	1.73 m	-0.01
13b	0.41 d (6.6)	0.42 d (6.5)	+0.01 (-0.1)
13c	0.89 m 1.22 m	0.89 m 1.22 m	0 0
13d	0.76 t (7.2)	0.76 t (7.3)	0 (+0.1)
15	7.92 d (9.6)	7.90 d (9.5)	-0.02 (-0.1)
16	4.70 m	4.72 m	+0.02
16a	2.59 m 2.80 m	2.59 m 2.80 m	0 0
16c	7.38 s	7.38 s	0
16e-OH	n.d.		
16g	7.38 s	7.38 s	0
18	8.53 d (10.0)	8.52 d (9.9)	-0.01 (-0.1)
19	4.63 m	4.63 m	0
19a-OH	5.14 s	5.11 s	-0.03
19b	1.10 s	1.11 s	+0.01
19c	1.02 s	1.02 s	0

4. X-Ray Crystallographic Data for Nannocystin Ax



Nannocystin Ax(**1**)

≡



X-ray structure of Nannocystin Ax(**1**)

Structure deposited at the Cambridge Crystallographic Data Centre (CCDC 1519855)

Crystal data and structure refinement for CCDC 1519855

Empirical formula	$C_{42}H_{55}Cl_2N_3O_8$
Formula weight	800.79
Temperature/K	293.15
Crystal system	monoclinic
Space group	$P2_1$
a/Å	14.4214(10)
b/Å	11.6137(6)
c/Å	16.9862(11)
α/°	90
β/°	111.966(8)
γ/°	90
Volume/Å^3	2638.4(3)
Z	2
ρ_{calc}g/cm^3	1.008
m/mm^{-1}	0.166
F(000)	852.0
Crystal size/mm^3	0.35 × 0.15 × 0.15
Radiation	MoKα (λ = 0.71073)
2Θ range for data collection/°	5.846 to 52.744
Index ranges	$-17 \le h \le 18$, $-14 \le k \le 9$, $-20 \le l \le 21$
Reflections collected	12198
Independent reflections	7779 [R_{int} = 0.0210, R_{sigma} = 0.0530]
Data/restraints/parameters	7779/3/521
Goodness-of-fit on F^2	0.927
Final R indexes [I>=2σ (I)]	R1 = 0.0587, wR2 = 0.1578
Final R indexes [all data]	R1 = 0.0881, wR2 = 0.1729
Largest diff. peak/hole / e Å^{-3}	0.27/-0.19

Bond lengths (Å) and bond angles (deg) for Nannocystin **Ax**

Bond length			
O1–C22	1.374(9)	C10–C35	1.455(9)
O2–C2	1.216(5)	C11–C12	1.512(8)
O4–C25	1.424(6)	C12–C13	1.488(8)
O5–C4	1.325(5)	C13–C14	1.307(8)
O5–C5	1.460(6)	C14–C15	1.492(7)
O6–C11	1.409(6)	C14–C37	1.506(9)
O6–C36	1.430(8)	C16–C17	1.529(7)
O7–C15	1.193(6)	C16–C39	1.538(7)
O8–C17	1.240(6)	C18–C19	1.540(8)
N1–C2	1.334(6)	C19–C20	1.401(8)
N1–C3	1.445(6)	C19–C24	1.382(8)
N2–C1	1.485(6)	C20–C21	1.344(9)
N2–C17	1.303(6)	C21–C22	1.375(10)
N3–C15	1.374(6)	C21–Cl1	1.745(8)
N3–C16	1.468(6)	C21–Cl1A	1.85(2)
N3–C38	1.442(7)	C22–C23	1.334(9)
C1–C2	1.503(6)	C23–C24	1.368(8)
C1–C18	1.525(7)	C23–Cl2	1.799(8)
C3–C4	1.508(7)	C23–Cl2A	1.689(19)
C3–C25	1.550(7)	C25–C26	1.520(8)
C4–O3	1.199(6)	C25–C27	1.491(8)
C4–O3A	1.17(4)	C28–C29	1.393(9)
C5–C6	1.537(7)	C28–C33	1.351(9)
C5–C28	1.515(7)	C29–C30	1.346(10)
C6–C7	1.481(7)	C30–C31	1.380(14)
C6–C34	1.506(7)	C31–C32	1.431(14)
C7–C8	1.309(7)	C32–C33	1.393(11)
C8–C9	1.473(7)	C39–C40	1.551(9)
C9–C10	1.321(7)	C39–C41	1.501(9)
C10–C11	1.530(8)	C41–C42	1.527(10)
Bond angle			
C4–O5–C5	117.3(4)	N3–C16–C17	107.9(4)
C11–O6–C36	113.0(5)	N3–C16–C39	113.3(4)
C2–N1–C3	122.4(4)	C17–C16–C39	113.7(4)
C17–N2–C1	121.8(5)	O8–C17–N2	123.8(5)
C15–N3–C16	118.1(4)	O8–C17–C16	120.3(4)
C15–N3–C38	122.9(4)	N2–C17–C16	115.9(4)
C38–N3–C16	118.1(4)	C1–C18–C19	116.6(4)
N2–C1–C2	106.2(4)	C20–C19–C18	119.1(5)
N2–C1–C18	111.4(4)	C24–C19–C18	122.6(5)
C2–C1–C18	114.3(4)	C24–C19–C20	118.3(6)
O2–C2–N1	122.8(4)	C21–C20–C19	119.8(6)
O2–C2–C1	121.3(4)	C20–C21–C22	122.7(6)
N1–C2–C1	115.8(4)	C20–C21–Cl1	118.3(7)
N1–C3–C4	108.3(4)	C20–C21–Cl1A	108.3(16)
N1–C3–C25	112.9(4)	C22–C21–Cl1	119.0(6)

C4–C3–C25	113.9(4)	C22–C21–Cl1A	127.1(17)
O5–C4–C3	111.8(4)	O1–C22–C21	125.3(7)
O3–C4–O5	125.0(5)	C23–C22–O1	118.5(7)
O3–C4–C3	123.2(5)	C23–C22–C21	116.2(7)
O3A–C4–O5	95(2)	C22–C23–C24	124.9(7)
O3A–C4–C3	150(2)	C22–C23–Cl2	120.2(6)
O5–C5–C6	104.8(4)	C22–C23–Cl2A	103.0(13)
O5–C5–C28	109.9(4)	C24–C23–Cl2	114.9(5)
C28–C5–C6	113.8(4)	C24–C23–Cl2A	132.0(13)
C7–C6–C5	109.2(4)	C23–C24–C19	118.1(5)
C7–C6–C34	117.1(5)	O4–C25–C3	108.1(4)
C34–C6–C5	113.0(4)	O4–C25–C26	109.7(4)
C8–C7–C6	126.6(5)	O4–C25–C27	106.7(5)
C7–C8–C9	122.0(5)	C26–C25–C3	109.2(4)
C10–C9–C8	127.7(5)	C27–C25–C3	111.8(4)
C9–C10–C11	119.5(5)	C27–C25–C26	111.2(5)
C9–C10–C35	124.1(5)	C29–C28–C5	118.1(6)
C35–C10–C11	116.2(5)	C33–C28–C5	123.9(5)
O6–C11–C10	111.4(4)	C33–C28–C29	117.8(5)
O6–C11–C12	105.5(4)	C30–C29–C28	124.2(8)
C12–C11–C10	114.3(5)	C29–C30–C31	118.1(9)
C13–C12–C11	115.5(5)	C30–C31–C32	119.9(8)
C14–C13–C12	125.7(5)	C33–C32–C31	118.4(9)
C13–C14–C15	120.7(5)	C28–C33–C32	121.4(7)
C13–C14–C37	124.2(6)	C16–C39–C40	107.7(5)
C15–C14–C37	115.1(5)	C41–C39–C16	111.2(5)
O7–C15–N3	122.3(5)	C41–C39–C40	114.2(5)
O7–C15–C14	121.3(4)	C39–C41–C42	115.8(7)
N3–C15–C14	116.3(4)		

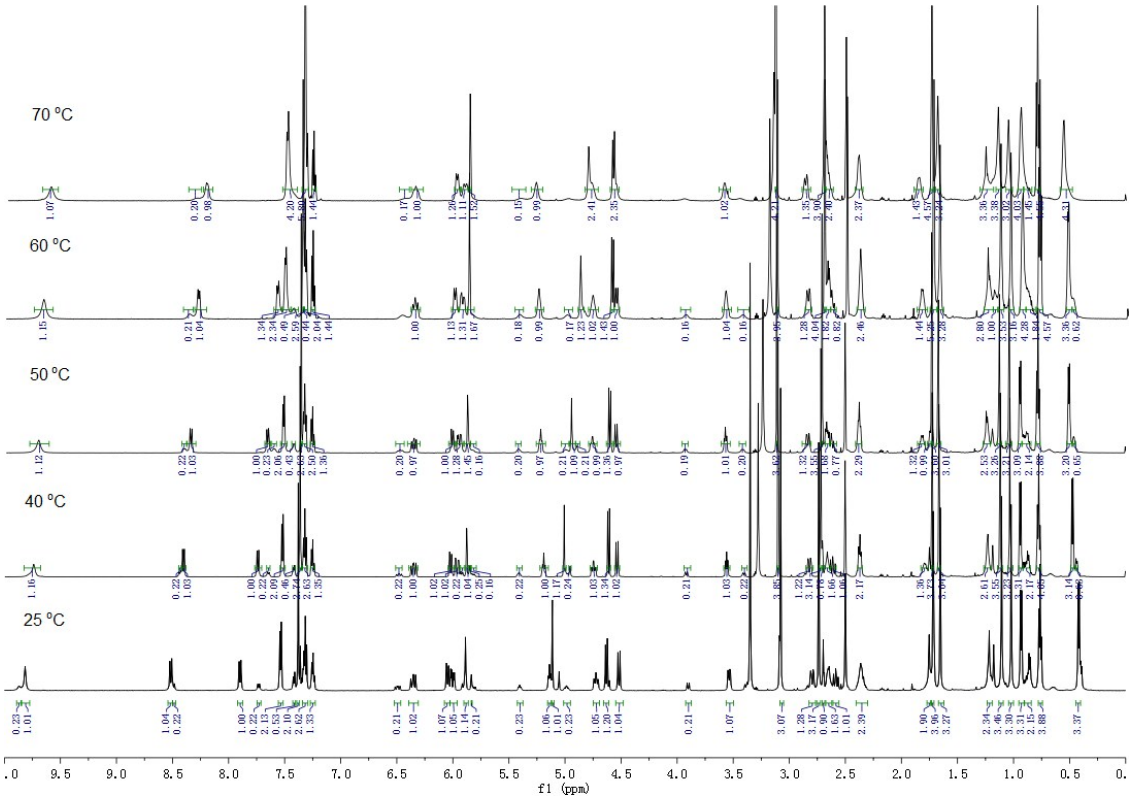
Torsion angles (deg) for Nannocystin **Ax**

O1–C22–C23–C24	-178.8(6)	C15–N3–C16–C17	-96.7(5)
O1–C22–C23–Cl2	2.3(8)	C15–N3–C16–C39	136.5(4)
O1C22C23Cl2A	5.6(13)	C16–N3–C15–O7	9.5(7)
O5–C5–C6–C7	-68.2(5)	C16–N3–C15–C14	-167.0(4)
O5–C5–C6–C34	64.0(5)	C16–C39–C41–C42	175.2(6)
O5–C5–C28–C29	166.5(5)	C17–N2–C1–C2	152.4(4)
O5–C5–C28–C33	-17.2(7)	C17–N2–C1–C18	-82.5(6)
O6–C11–C12–C13	168.7(5)	C17–C16–C39–C40	61.0(6)
N1–C3–C4–O5	151.7(4)	C17–C16–C39–C41	-173.2(5)
N1–C3–C4–O3	-27.1(7)	C18–C1–C2–O2	-115.8(5)
N1–C3–C4–O3A	-56(5)	C18–C1–C2–N1	62.3(6)
N1–C3–C25–O4	-175.4(4)	C18–C19–C20–C21	178.8(5)
N1–C3–C25–C26	-56.1(5)	C18–C19–C24–C23	-179.4(5)
N1–C3–C25–C27	67.3(6)	C19–C20–C21–C22	1.8(9)
N2–C1–C2–O2	7.4(6)	C19–C20–C21–Cl1	-180.0(5)

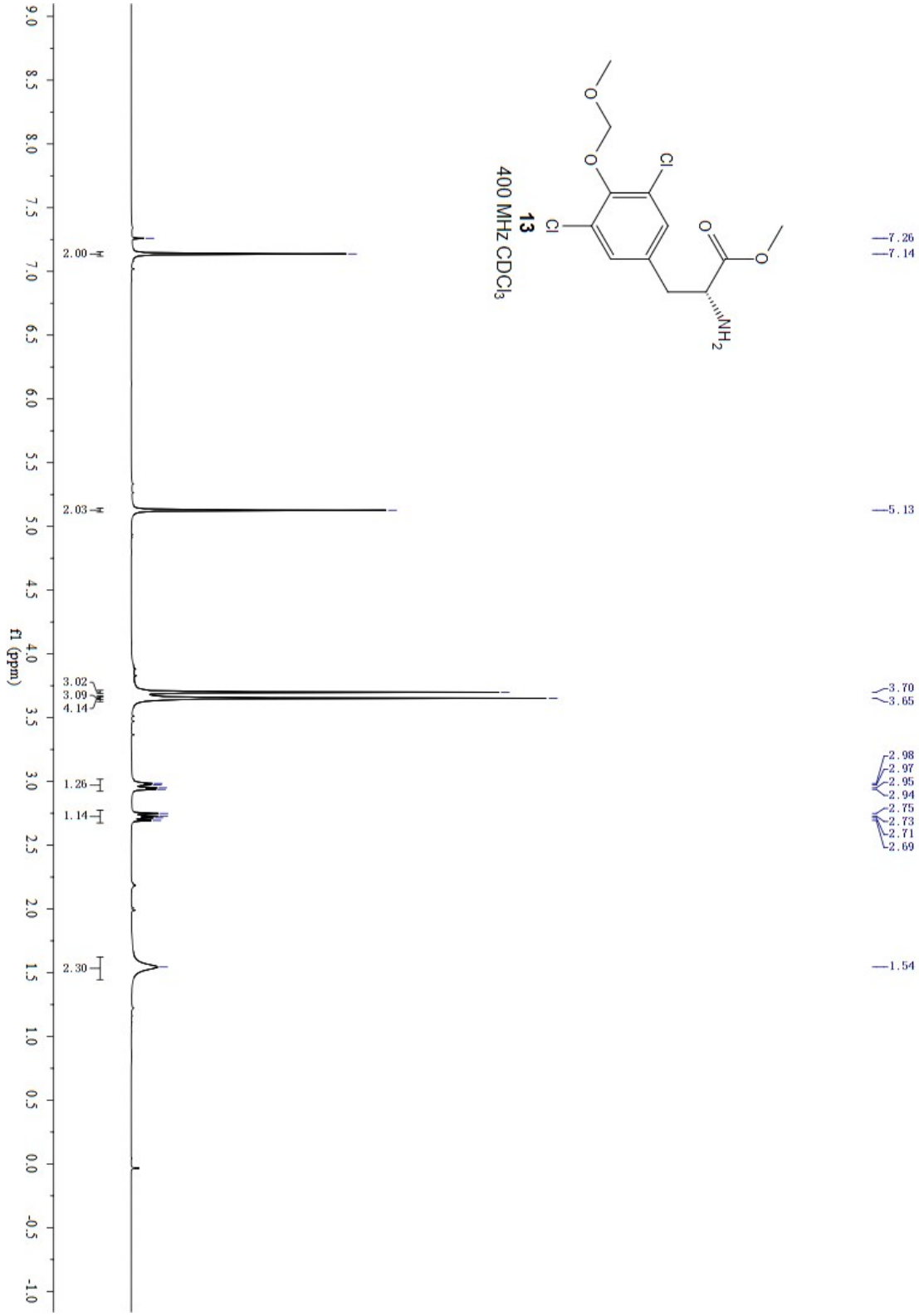
N2–C1–C–2N1	-174.4(4)	C19–C20–C21–Cl1A	-163.3(14)
N2–C1–C18–C19	-78.8(5)	C20–C19–C24–C23	0.5(8)
N3–C16–C17–O8	-104.8(5)	C20–C21–C22–O1	178.2(6)
N3–C16–C17–N2	73.6(5)	C20–C21–C22–C23	-1.9(9)
N3–C16–C39–C40	-175.3(4)	C21–C22–C23–C24	1.3(9)
N3–C16–C39–C41	-49.5(6)	C21–C22–C23–Cl2	-177.7(5)
C1–N2–C17–O8	6.8(8)	C21–C22–C23–Cl2A	-174.4(13)
C1–N2–C17–C16	-171.5(4)	C22–C23–C24–C19	-0.6(9)
C1–C18–C19–C20	-130.0(5)	C24–C19–C20–C21	-1.0(8)
C1–C18–C19–C24	49.8(7)	C25–C3–C4–O5	-81.7(5)
C2–N1–C3–C4	-120.6(4)	C25–C3–C4–O3	99.5(6)
C2–N1vC3–C25	112.2(5)	C25–C3–C4–O3A	70(5)
C2–C1–C18vC19	41.6(6)	C28–C5–C6–C7	171.7(4)
C3–N1–C2–O2	-7.6(7)	C28–C5–C6–C34	-56.1(6)
C3–N1–C2–C1	174.3(4)	C28–C29–C30–C31	0.7(13)
C4–O5–C5–C6	144.7(4)	C29–C28–C33–C32	0.4(10)
C4–O5–C5–C28	-92.7(5)	C29–C30–C31–C32	-2.6(14)
C4–C3–C25–O4	60.4(5)	C30–C31–C32–C33	3.4(15)
C4–C3–C25–C26	179.7(4)	C31–C32–C33–C28	-2.3(13)
C4–C3–C25–C27	-56.8(6)	C33–C28–C29–C30	0.5(10)
C5–O5–C4–C3	-177.1(4)	C34–C6–C7–C8	5.6(8)
C5–O5–C4–O3	1.7(7)	C35–C10–C11–O6	56.0(7)
C5–O5–C4–O3A	17(2)	C35–C10–C11–C12	-63.4(7)
C5–C6–C7–C8	135.7(5)	C36–O6–C11–C10	64.4(6)
C5–C28–C29–C30	177.0(7)	C36–O6–C11–C12	-171.1(5)
C5–C28–C33–C32	-176.0(7)	C37–C14–C15–O7	-117.0(6)
C6–C5–C28–C29	-76.3(6)	C37–C14–C15–N3	59.6(7)
C6–C5–C28–C33	100.0(6)	C38–N3–C15–O7	-159.4(5)
C6–C7–C8–C9	177.9(5)	C38–N3–C15–C14	24.1(7)
C7–C8–C9–C10	-178.4(5)	C38–N3–C16–C17	72.7(5)
C8–C9–C10–C11	178.6(5)	C38–N3–C16–C39	-54.0(6)
C8–C9–C10–C35	2.7(10)	C39–C16–C17–O8	21.8(7)
C9–C10–C11–O6	-120.2(5)	C39–C16–C17–N2	-159.9(5)
C9–C10–C11–C12	120.4(6)	C40–C39–C41–C42	-62.7(8)
C10–C11–C12–C13	-68.7(7)	Cl1–C21–C22–O1	0.0(9)
C11–C12–C13–C14	-132.1(6)	Cl1–C21–C22–C23	179.9(5)
C12–C13–C14–C15	-179.3(5)	Cl2–C23–C24–C19	178.4(4)
C12–C13–C14–C37	-0.3(10)	Cl1A–C21–C22–O1	-19.7(18)
C13–C14–C15–O7	62.1(7)	Cl1A–C21–C22–C23	160.2(16)
C13–C14–C15–N3	-121.3(5)	Cl2A–C23–C24–C19	173.7(16)

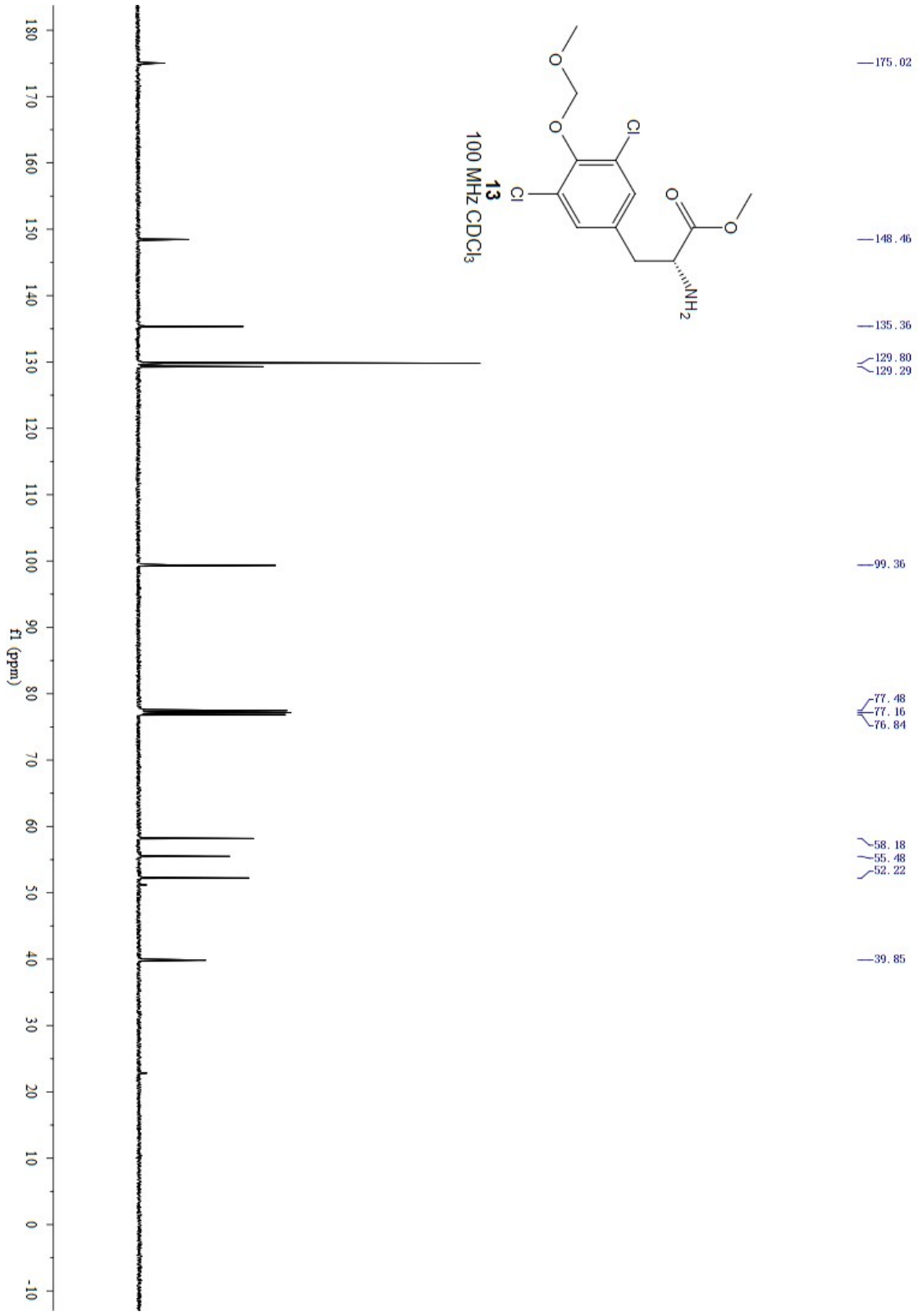
5. Variable temperature NMR experiment of Nannocystin Ax in DMSO-d_6

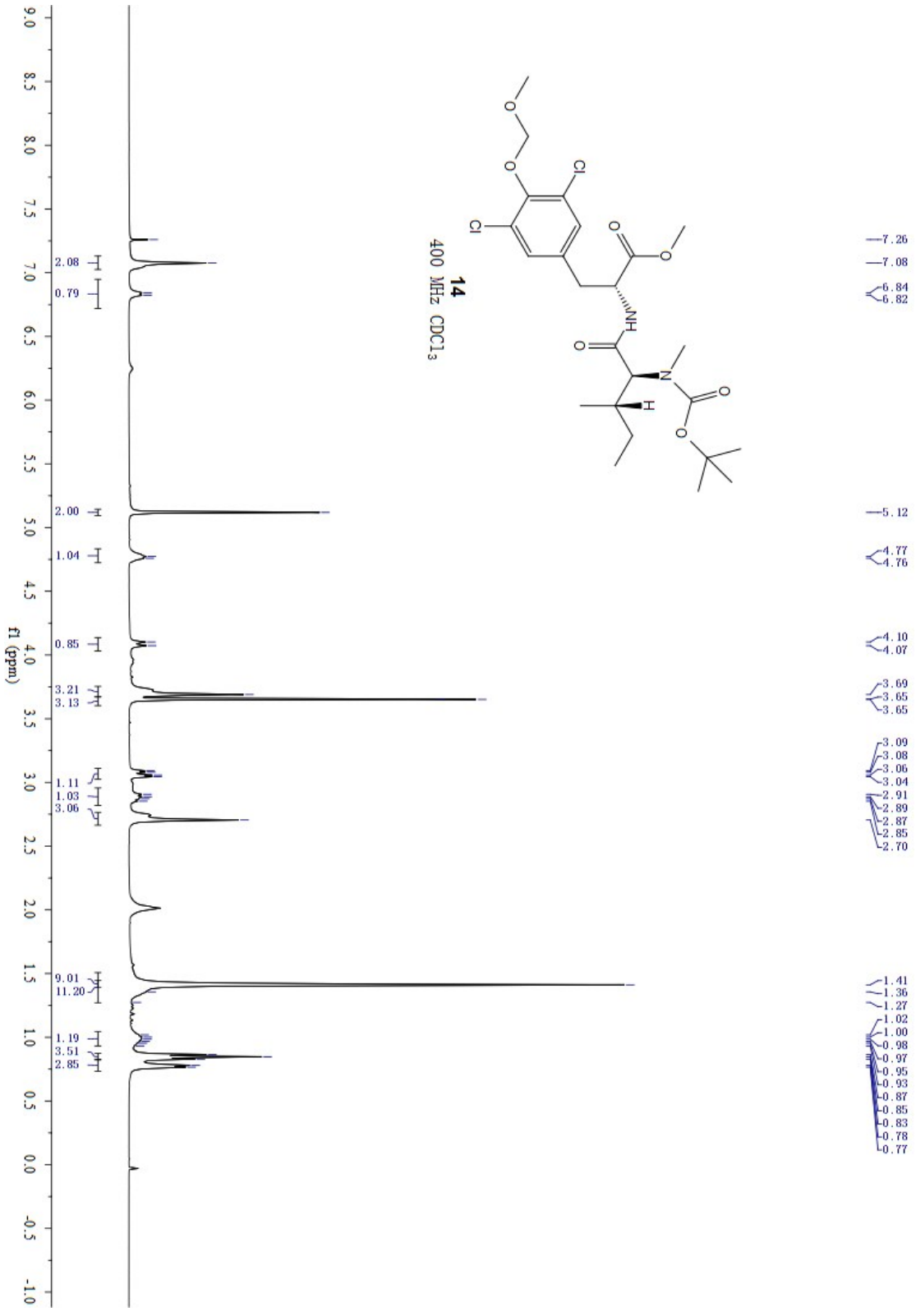
Nannocystin Ax (**1**) was selected to perform NMR experiments in DMSO-d_6 at temperatures ranging from 25 °C to 70°C. Temperature tunable NMR results proved that the NMR spectrum of 5:1 conformer of Nannocystin Ax (**1**) would appear in DMSO-d_6 at room temperature and the NMR peaks of isomers would be gradually overlapped with the increasing temperature.

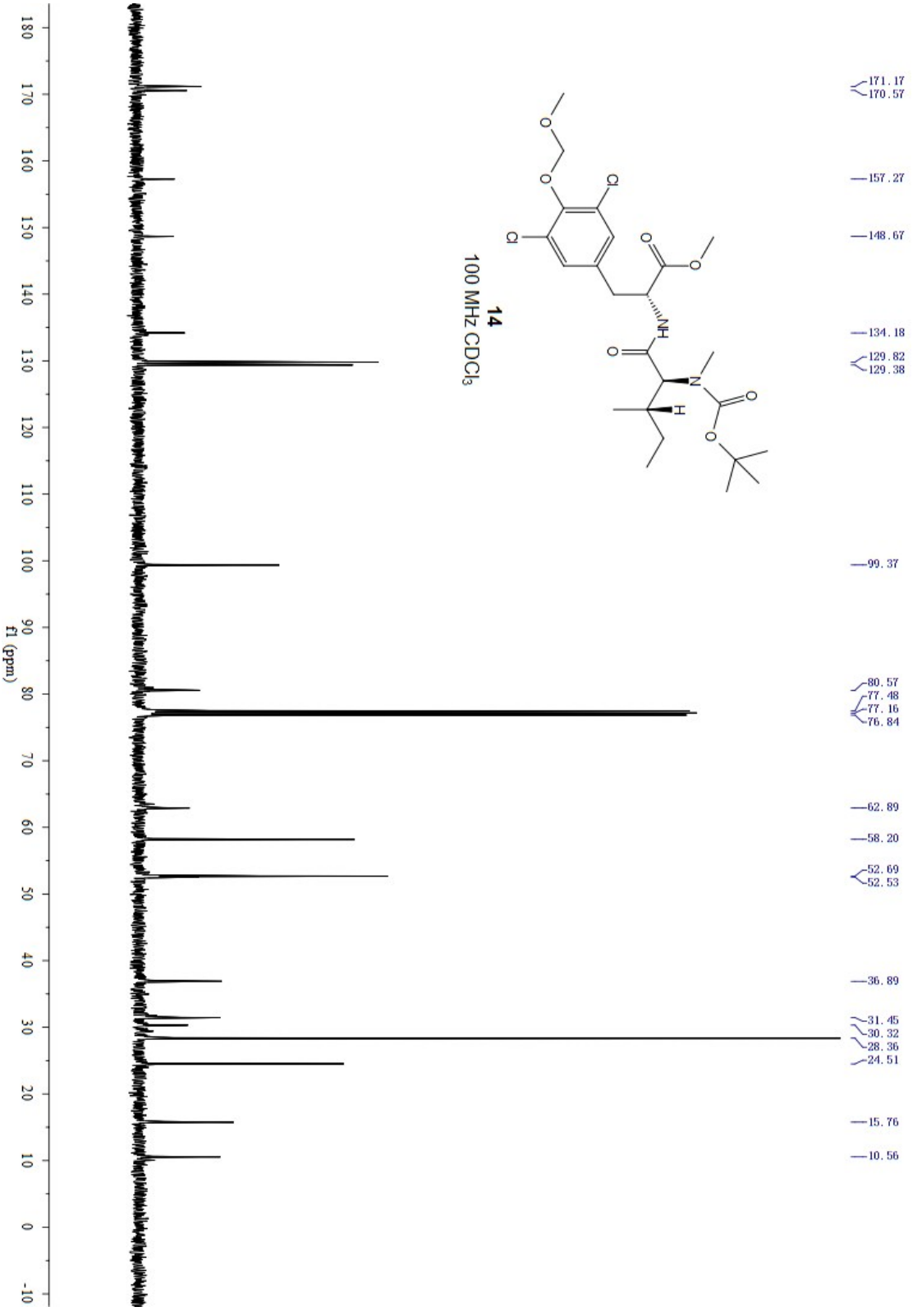


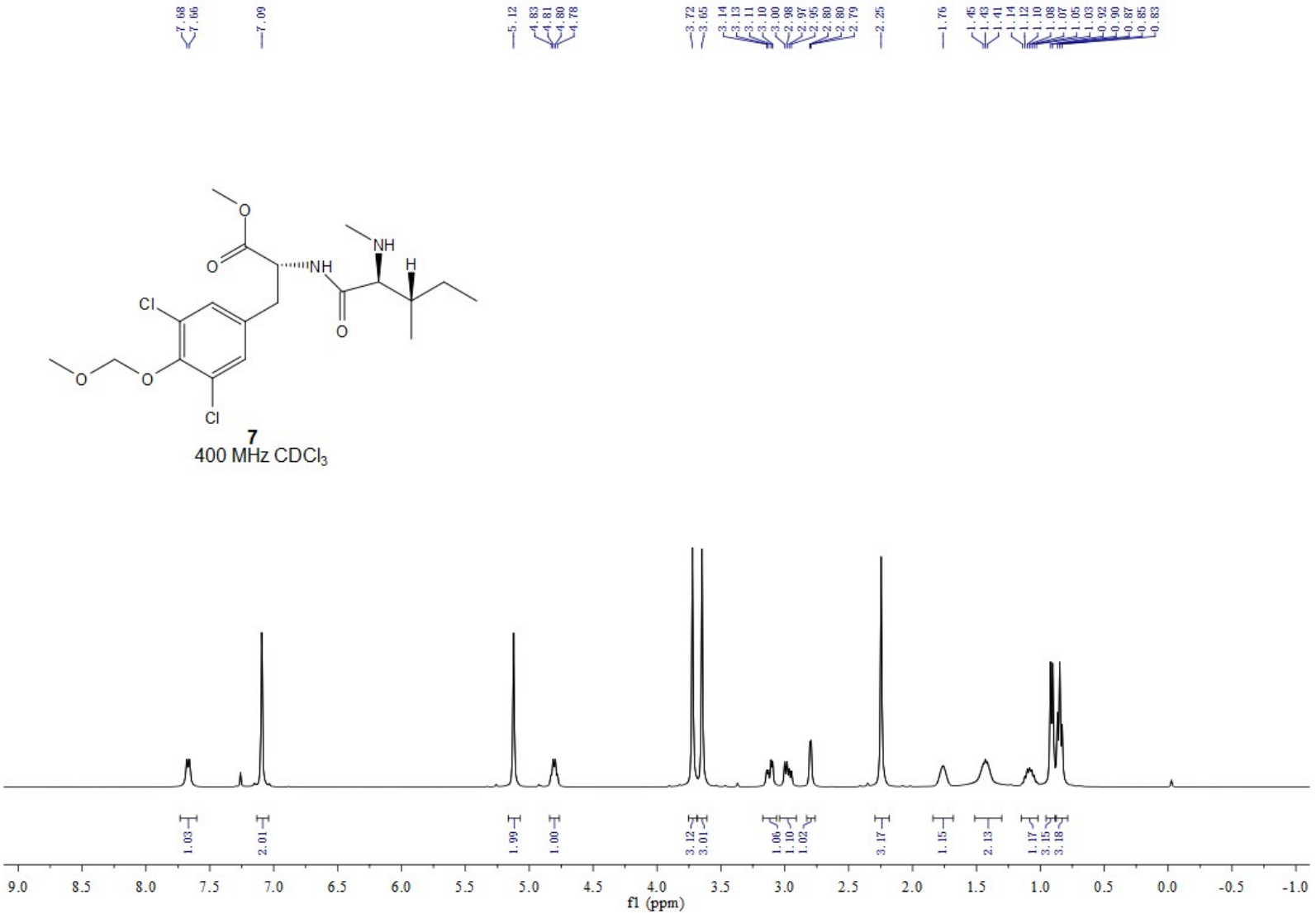
6. ^{1}H NMR and ^{13}C NMR Spectra

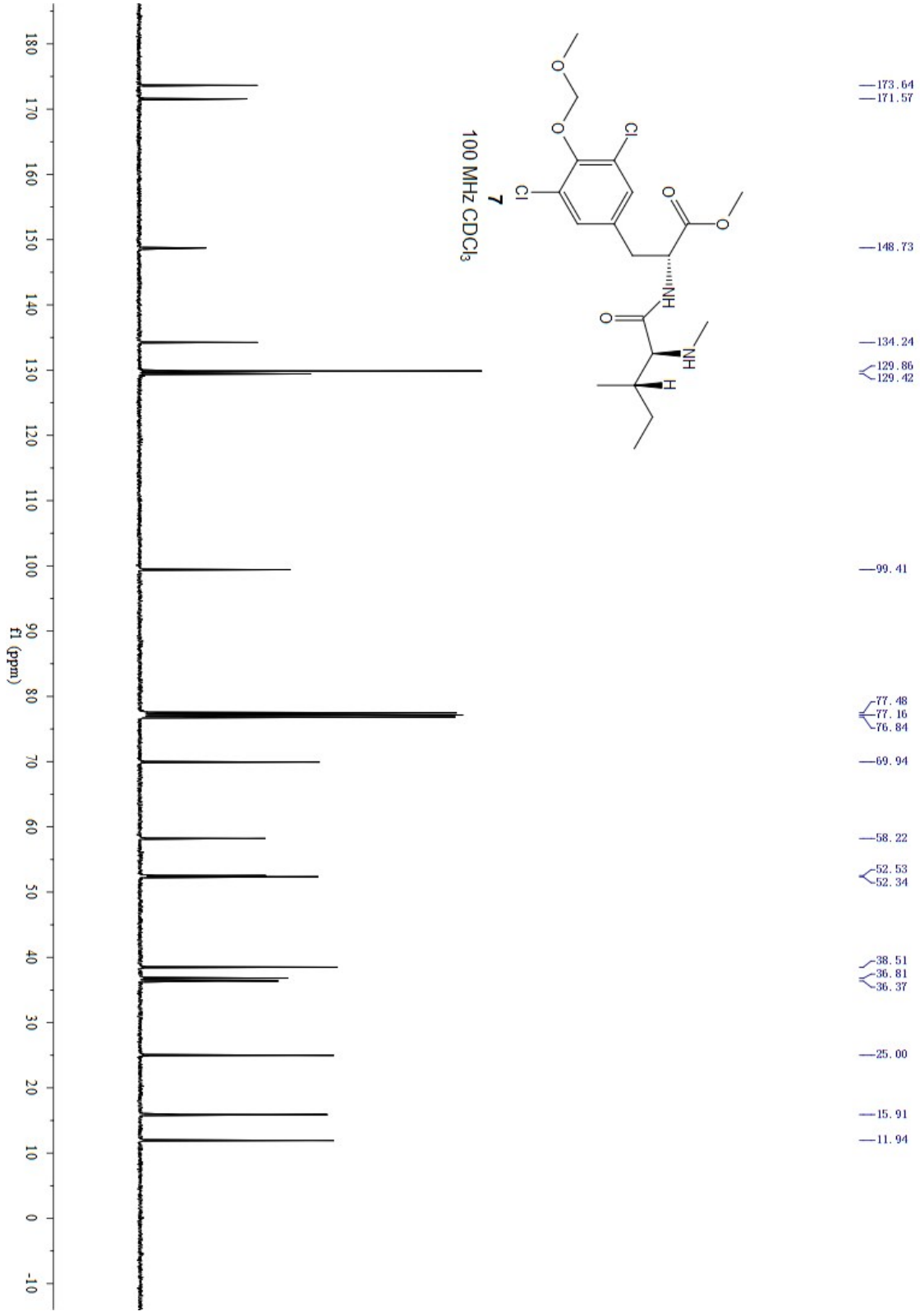


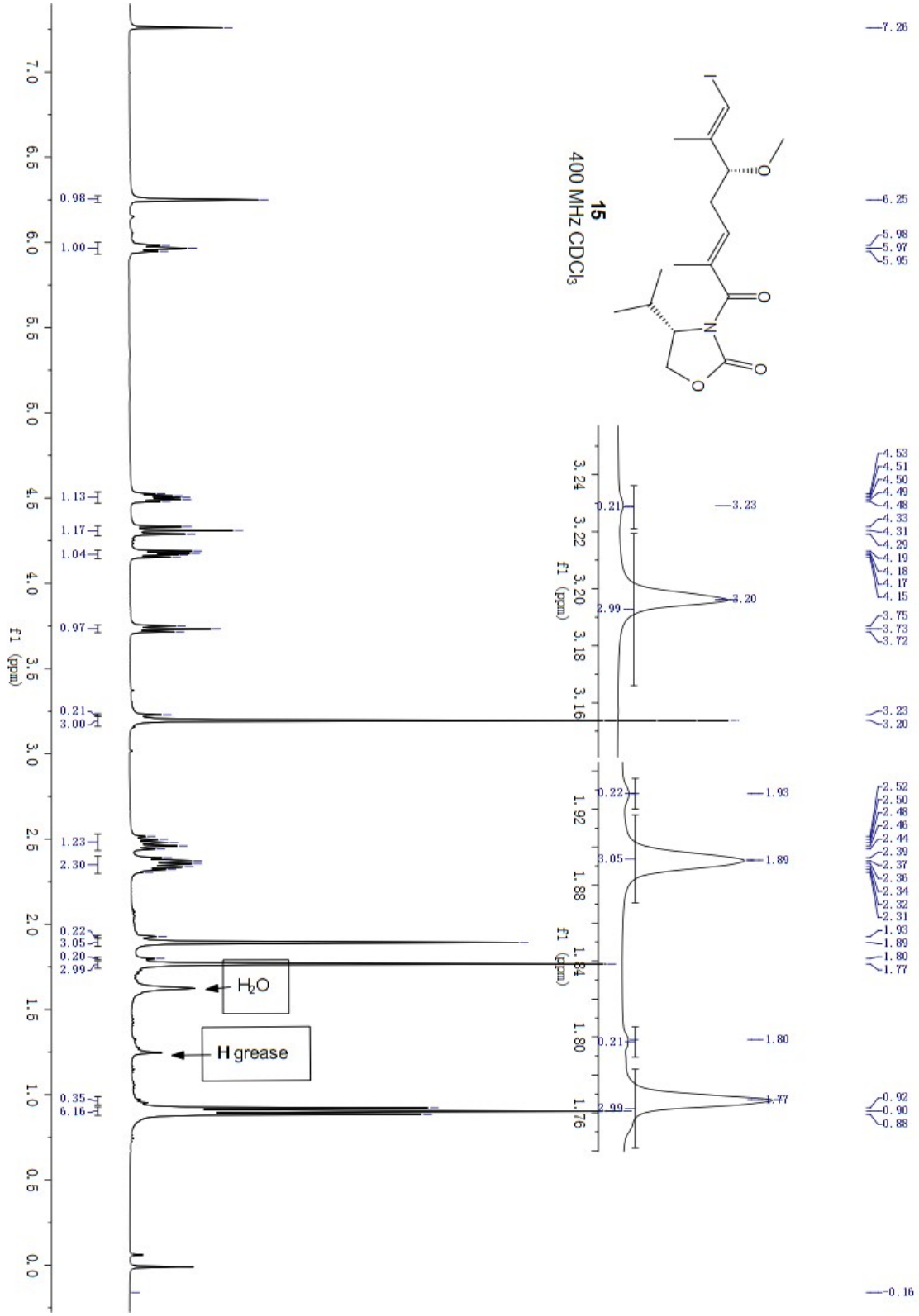

13
100 MHz CDCl3
Cl
Cl
O
O
O
O
NH2
175.02
148.46
135.36
129.80
129.29
99.36
77.48
77.16
76.84
58.18
55.48
52.22
39.85
f1 (ppm)

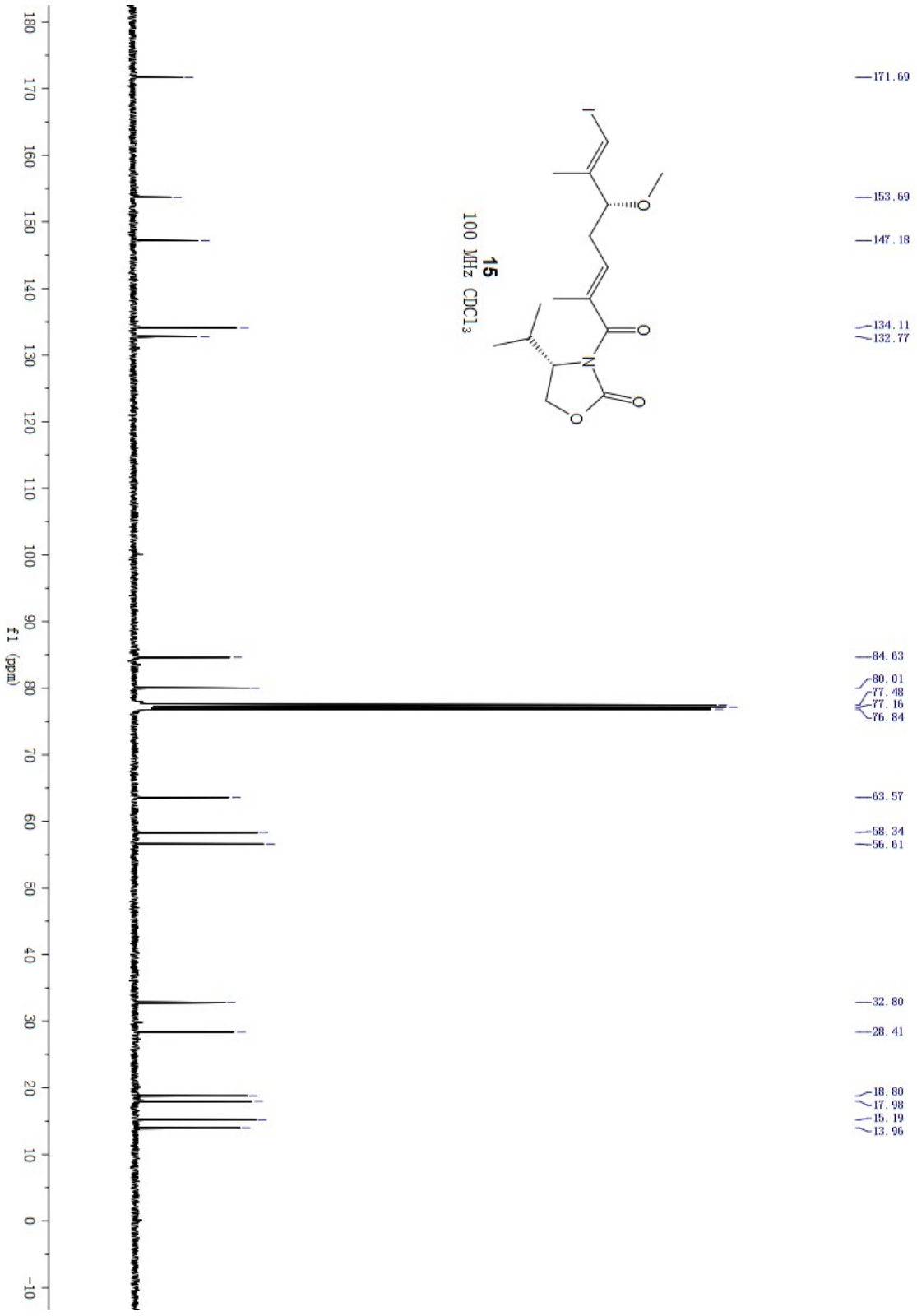

14
400 MHz CDCl3
f1 (ppm)
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
2.08
0.79
2.00
1.04
0.85
3.21
3.13
1.11
1.03
3.06
9.01
11.20
1.19
3.51
2.85
7.26
7.08
6.84
6.82
5.12
4.77
4.76
4.10
4.07
3.69
3.65
3.65
3.09
3.08
3.06
3.04
2.91
2.89
2.87
2.85
2.70
1.41
1.36
1.27
1.02
1.00
0.98
0.97
0.95
0.93
0.87
0.85
0.83
0.78
0.77

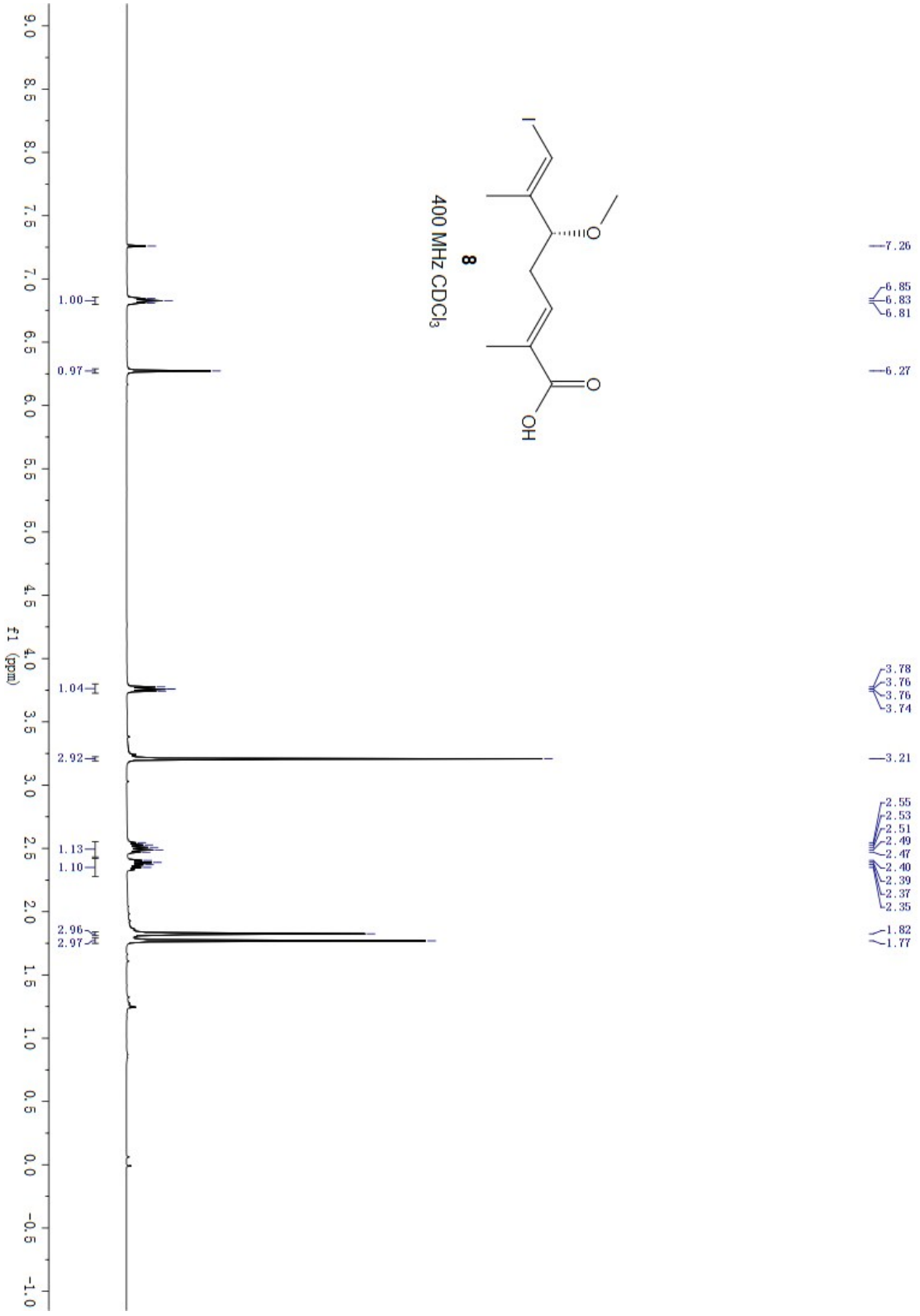

14
100 MHz CDCl3
171.17
170.57
157.27
148.67
134.18
129.82
129.38
99.37
80.57
77.48
77.16
76.84
62.89
58.20
52.69
52.53
36.89
31.45
30.32
28.36
24.51
15.76
10.56
f1 (ppm)

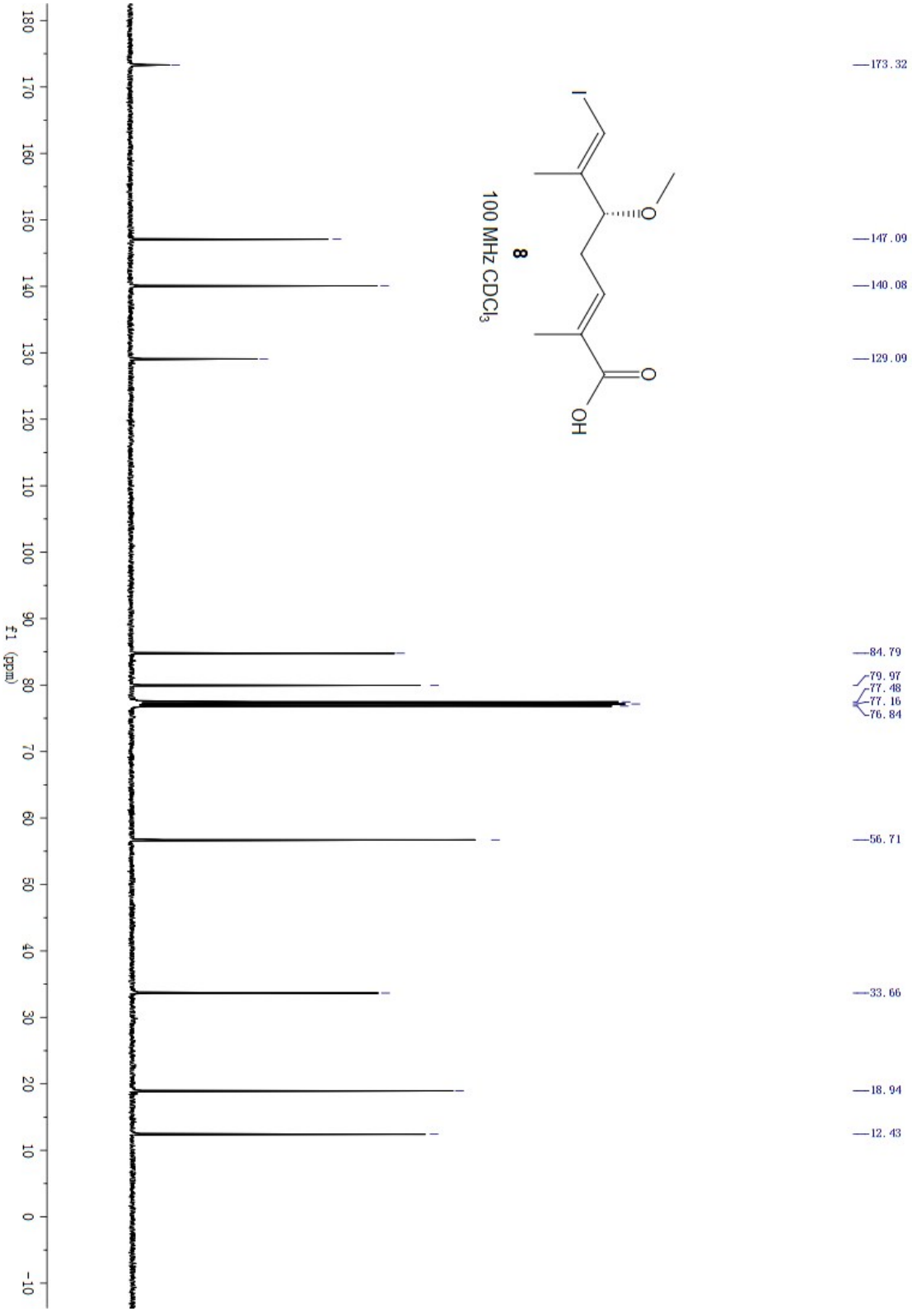

7.68
7.66
7.09
5.12
4.83
4.81
4.80
4.78
3.72
3.65
3.14
3.13
3.11
3.10
3.00
2.98
2.97
2.95
2.80
2.80
2.79
2.25
1.76
1.45
1.43
1.41
1.14
1.12
1.10
1.08
1.07
1.05
1.03
0.92
0.90
0.87
0.85
0.83
O
O
NH
NH
H
O
Cl
O
O
Cl
7
400 MHz CDCl3
1.03
2.01
1.99
1.00
3.12
3.01
1.06
1.10
1.02
3.17
1.15
2.13
1.17
3.15
3.18
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
f1 (ppm)

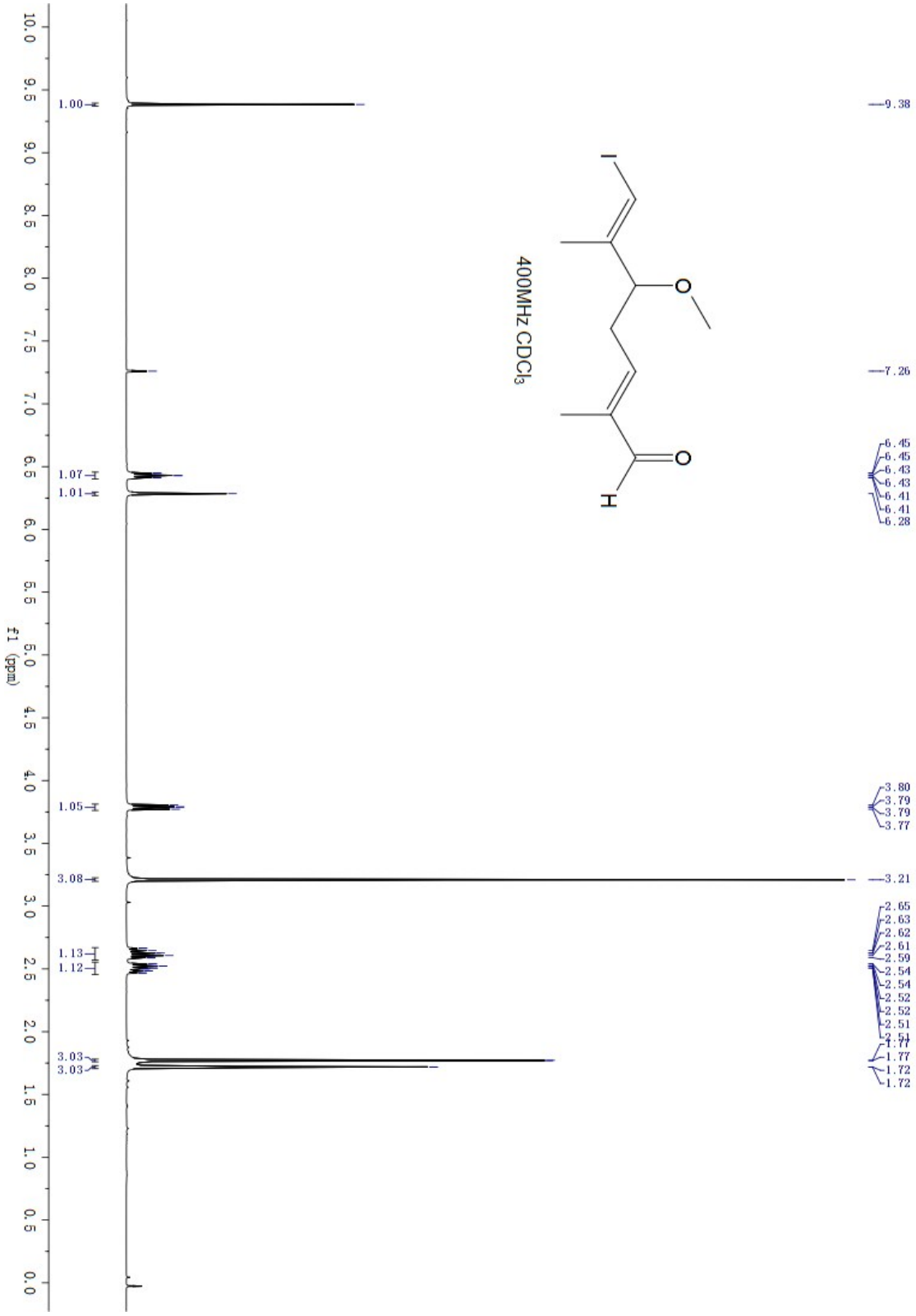

100 MHz CDCl3
7
f1 (ppm)
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
173.64
171.57
148.73
134.24
129.86
129.42
99.41
77.48
77.16
76.84
69.94
58.22
52.53
52.34
38.51
36.81
36.37
25.00
15.91
11.94

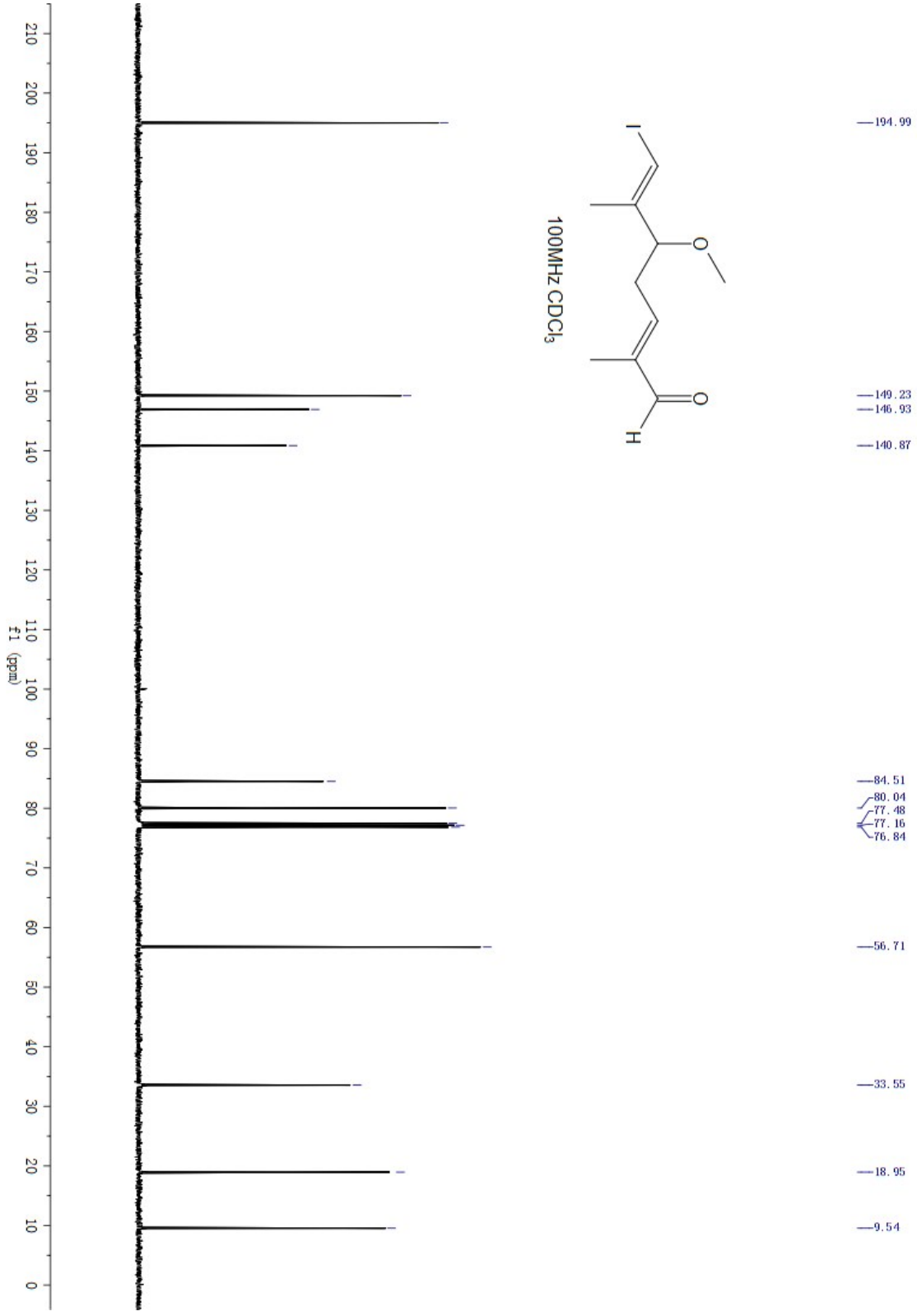

15
400 MHz CDCl3
H2O
H grease
f1 (ppm)

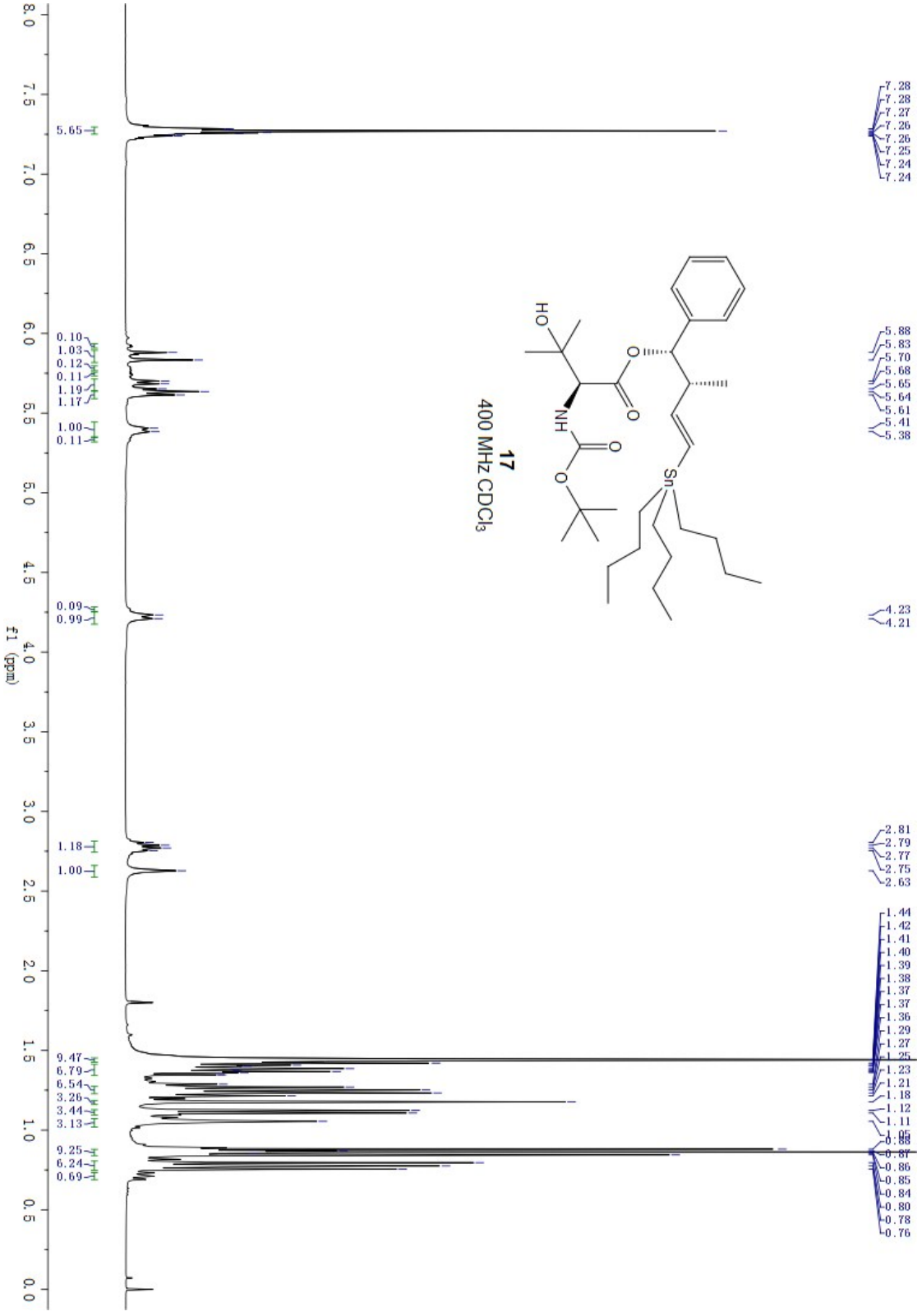

15
100 MHz CDCl3
171.69
153.69
147.18
134.11
132.77
84.63
80.01
77.48
77.16
76.84
63.57
58.34
56.61
32.80
28.41
18.80
17.98
15.19
13.96
f1 (ppm)

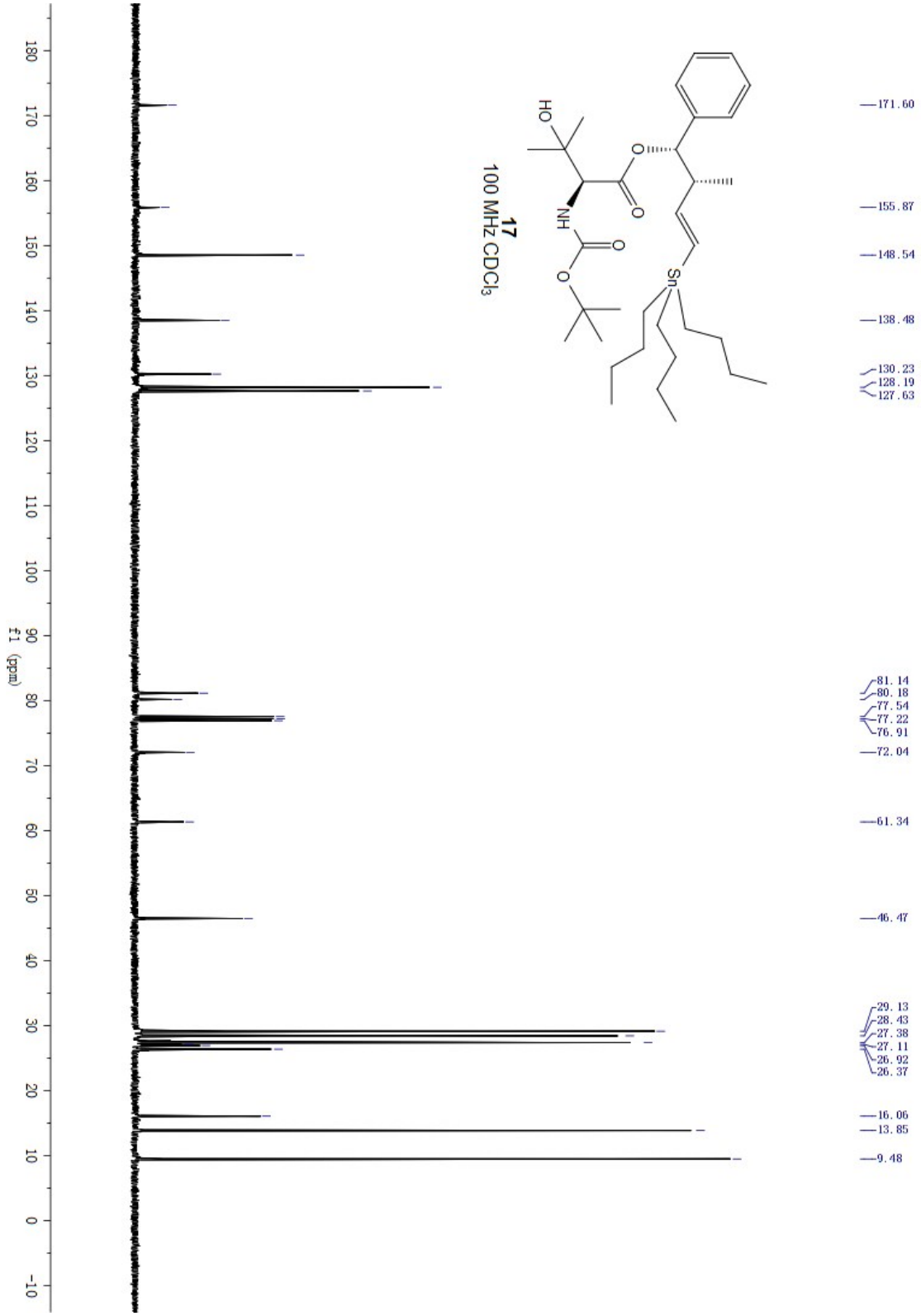

8
400 MHz CDCl3
I
O
OH
7.26
6.85
6.83
6.81
6.27
3.78
3.76
3.76
3.74
3.21
2.55
2.53
2.51
2.49
2.47
2.40
2.39
2.37
2.35
1.82
1.77
1.00
0.97
1.04
2.92
1.13
1.10
2.96
2.97
f1 (ppm)

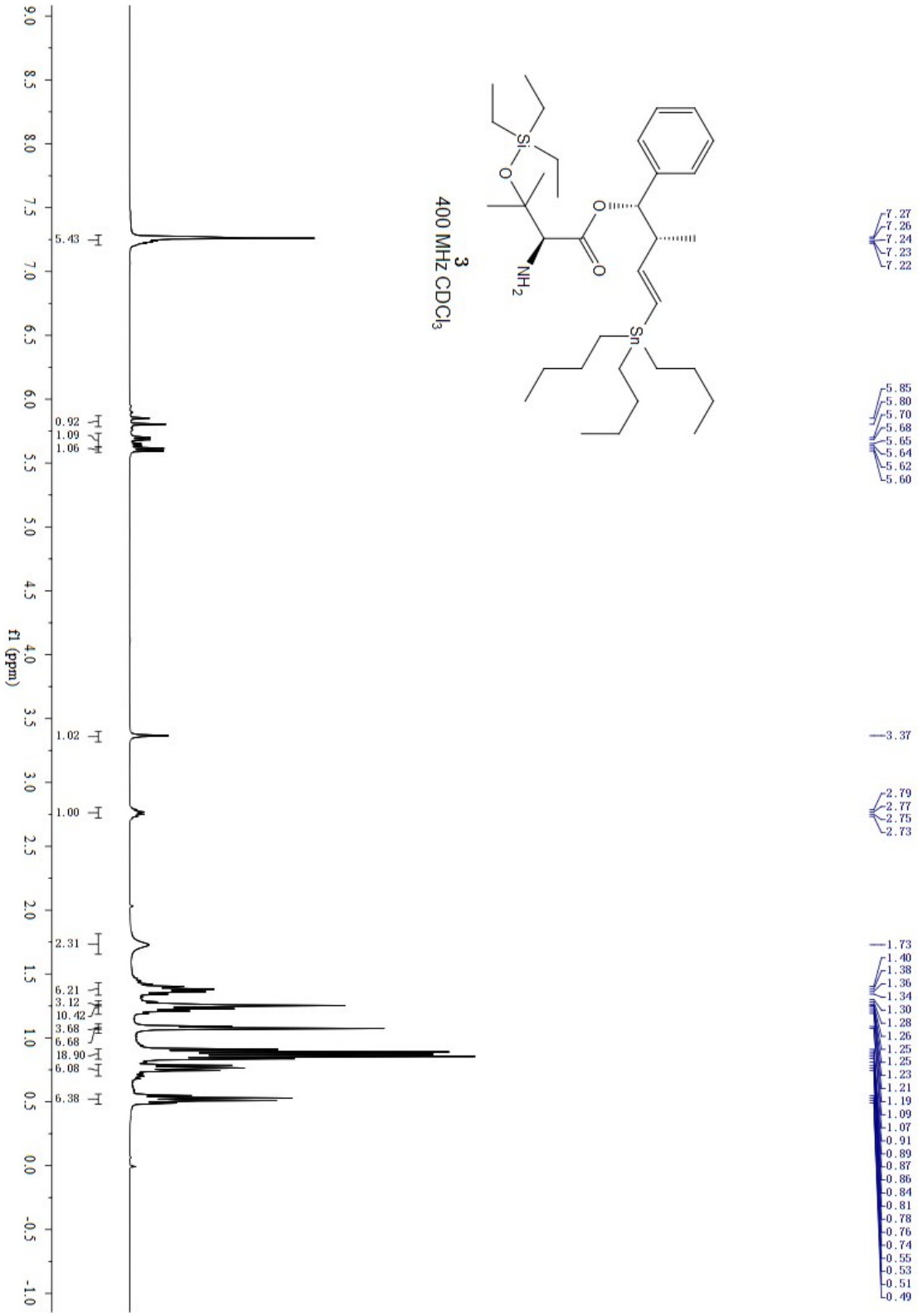

8
100 MHz CDCl3
I
O
O
OH
173.32
147.09
140.08
129.09
84.79
79.97
77.48
77.16
76.84
56.71
33.66
18.94
12.43
f1 (ppm)

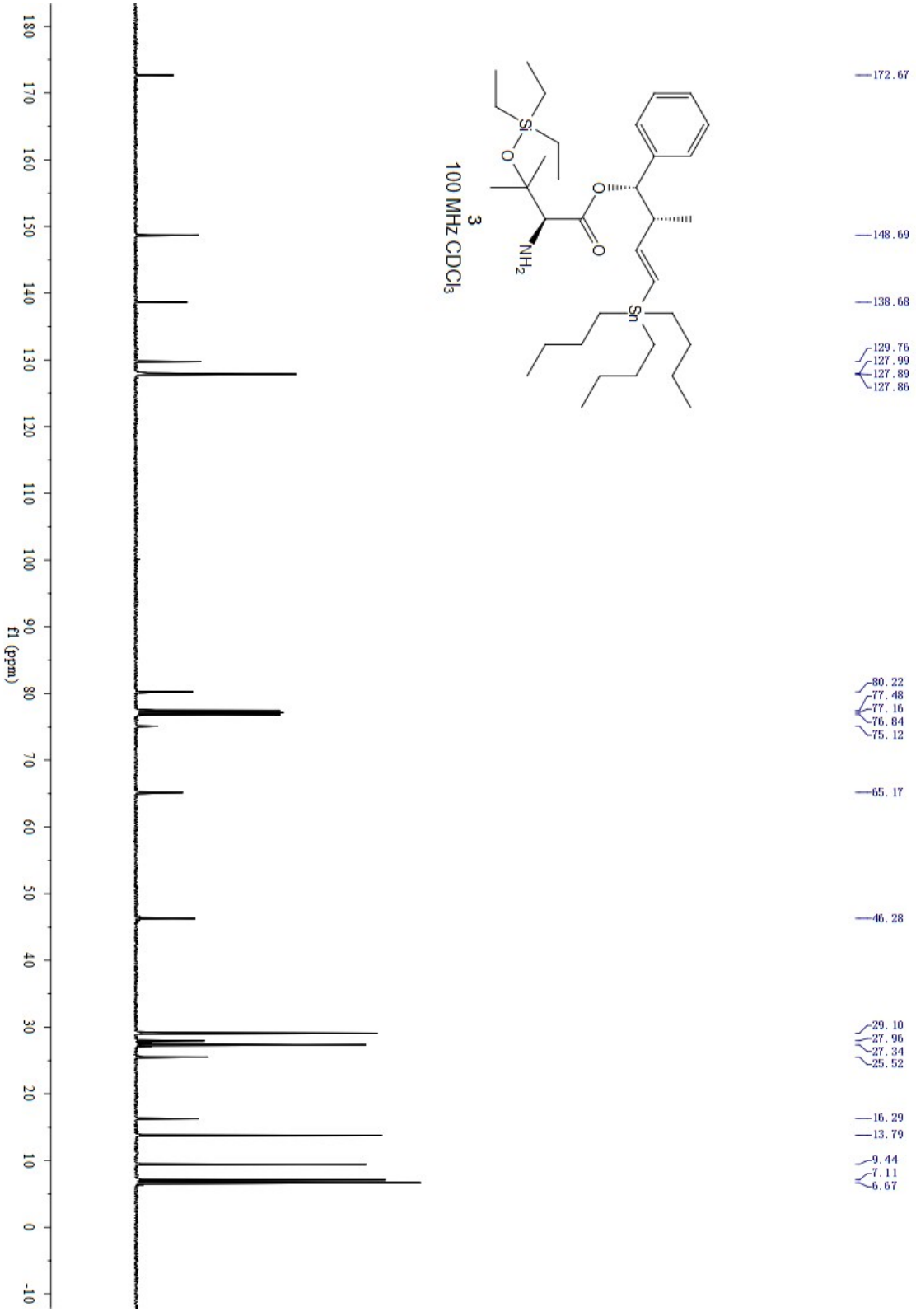

400MHz CDCl3
f1 (ppm)
1.00
1.07
1.01
1.05
3.08
1.13
1.12
3.03
3.03
9.38
7.26
6.45
6.45
6.43
6.43
6.41
6.41
6.28
3.80
3.79
3.79
3.77
3.21
2.65
2.63
2.62
2.61
2.59
2.54
2.54
2.52
2.52
2.51
1.77
1.77
1.72
1.72

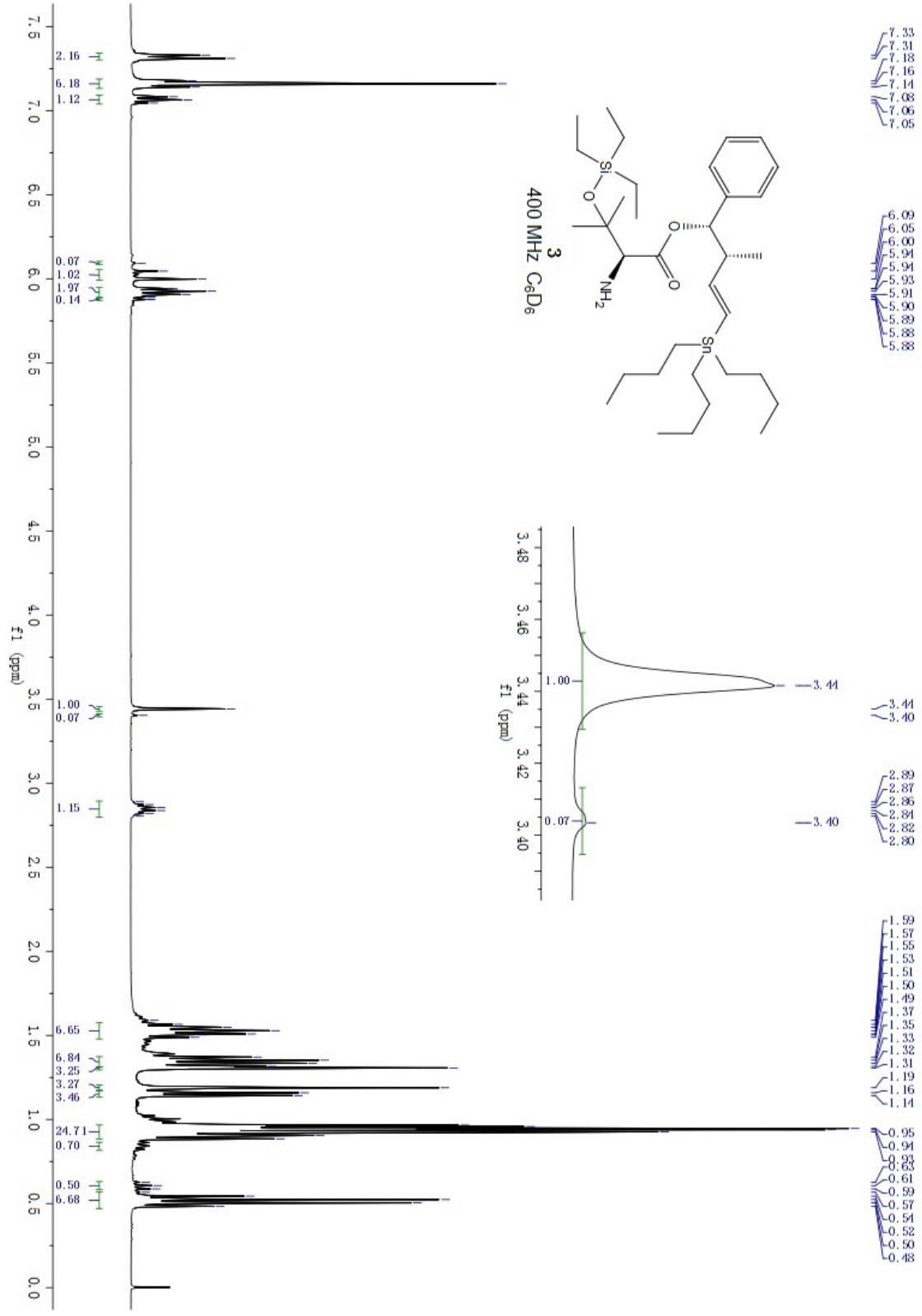

194.99
149.23
146.93
140.87
84.51
80.04
77.48
77.16
76.84
56.71
33.55
18.95
9.54
100MHz CDCl3
f1 (ppm)

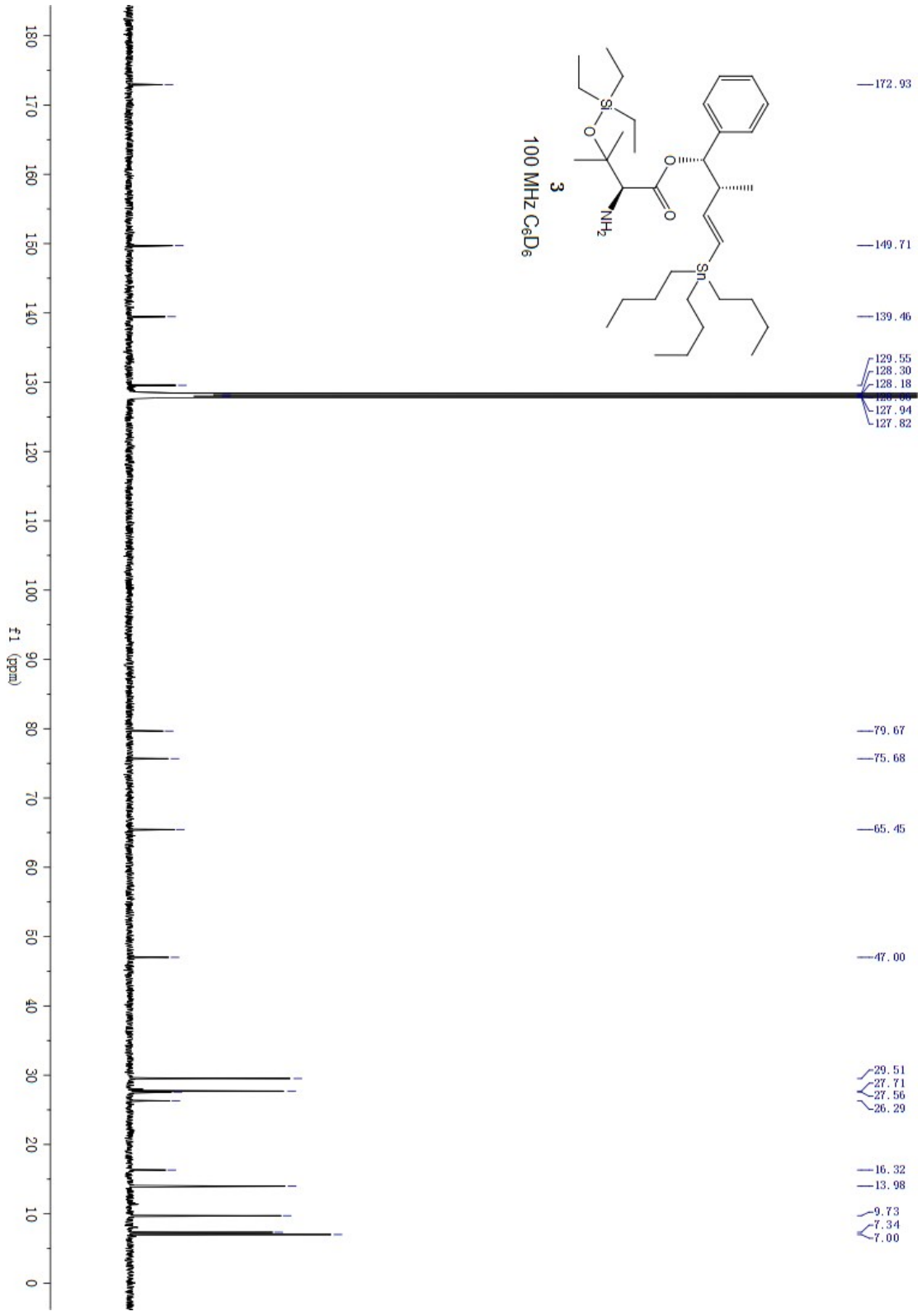

17
400 MHz CDCl3
f1 (ppm)

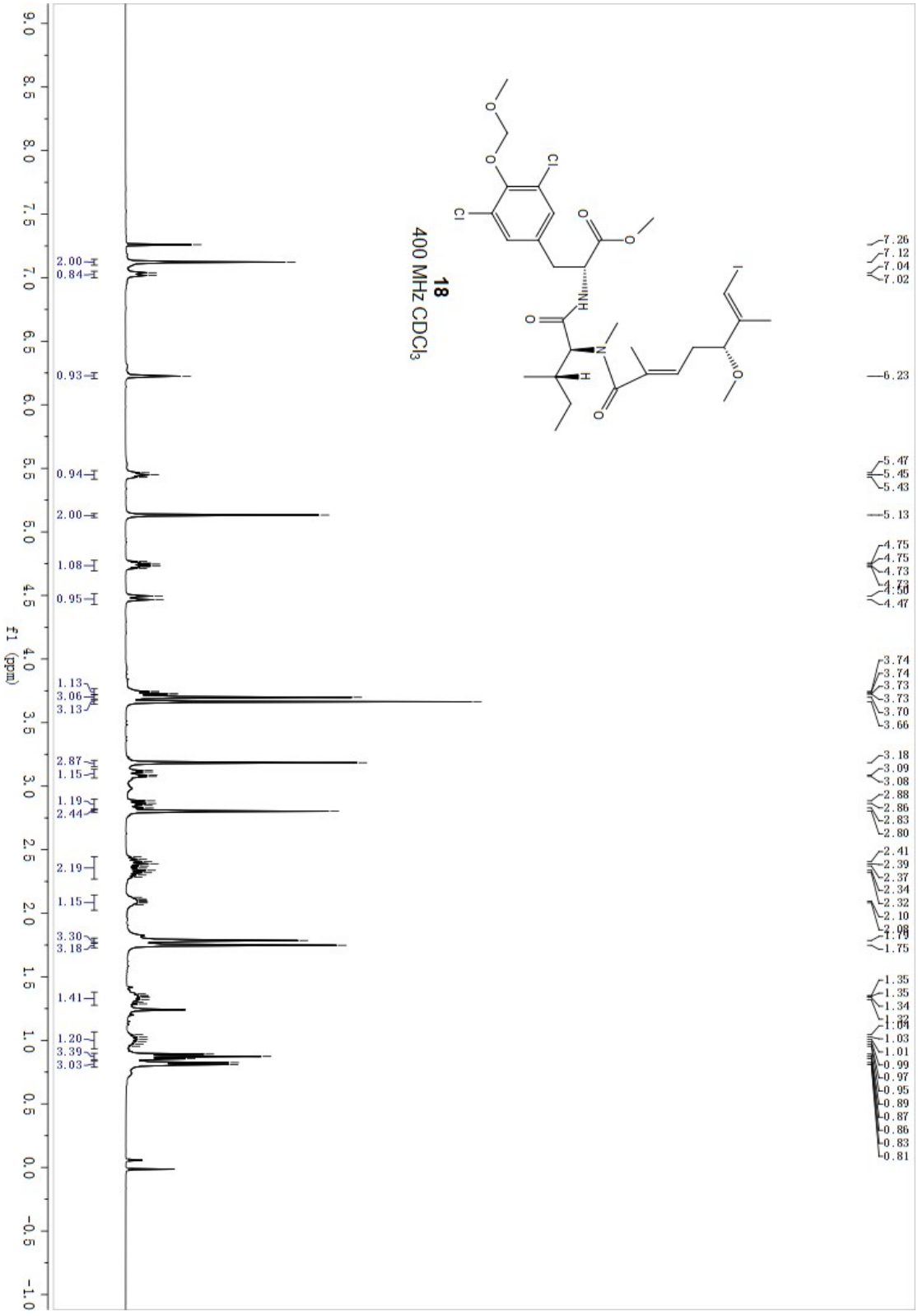

17
100 MHz CDCl3
HO
NH
O
Sn
171.60
155.87
148.54
138.48
130.23
128.19
127.63
81.14
80.18
77.54
77.22
76.91
72.04
61.34
46.47
29.13
28.43
27.38
27.11
26.92
26.37
16.06
13.85
9.48
f1 (ppm)

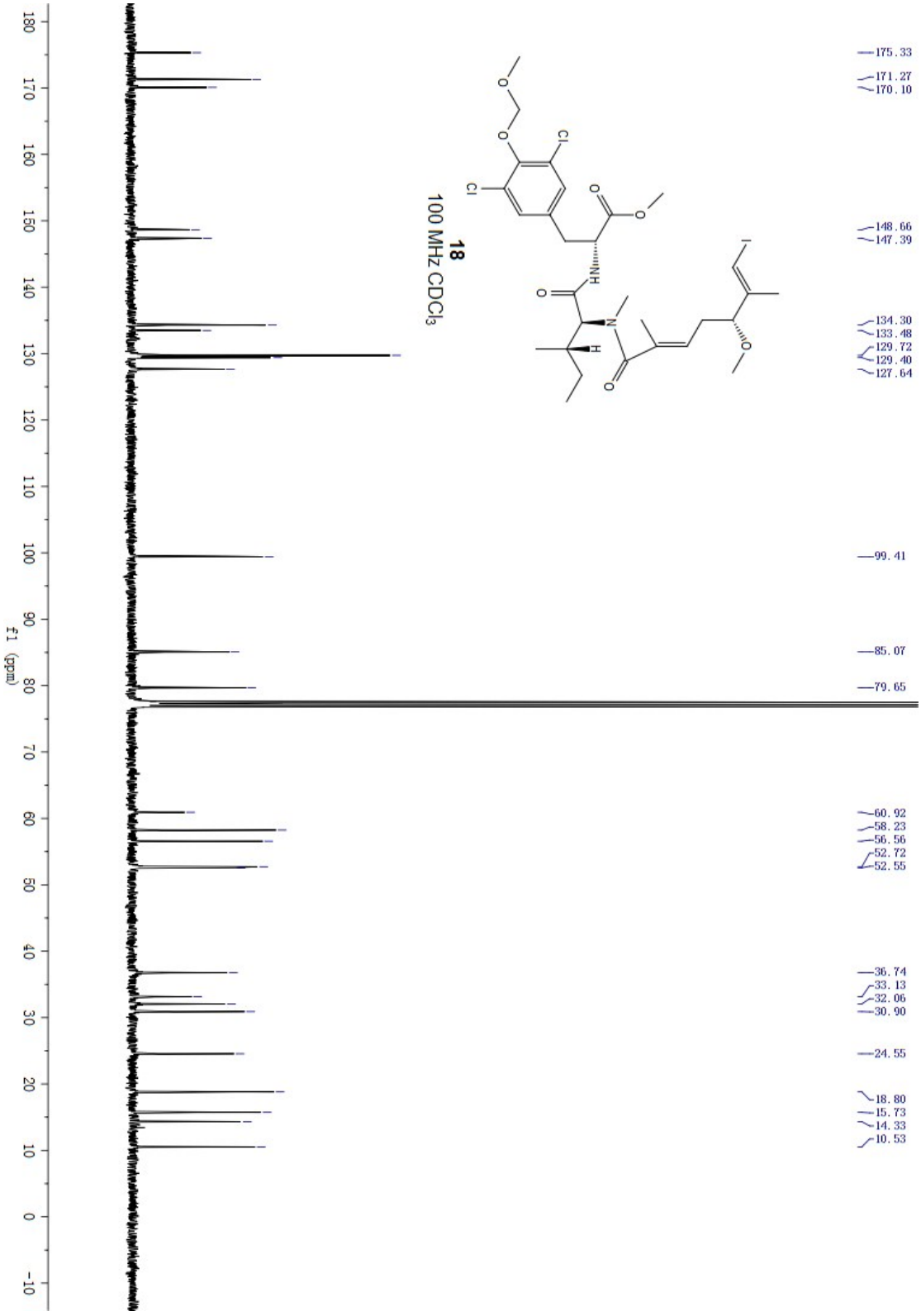

3
400 MHz CDCl3
f1 (ppm)
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
5.43
0.92
1.09
1.06
1.02
1.00
2.31
6.21
3.12
10.42
3.68
6.68
18.90
6.08
6.38
Si
O
NH2
O
O
Sn
7.27
7.26
7.24
7.23
7.22
5.85
5.80
5.70
5.68
5.65
5.64
5.62
5.60
3.37
2.79
2.77
2.75
2.73
1.73
1.40
1.38
1.36
1.34
1.30
1.28
1.26
1.25
1.25
1.23
1.21
1.19
1.09
1.07
0.91
0.89
0.87
0.86
0.84
0.81
0.78
0.76
0.74
0.55
0.53
0.51
0.49

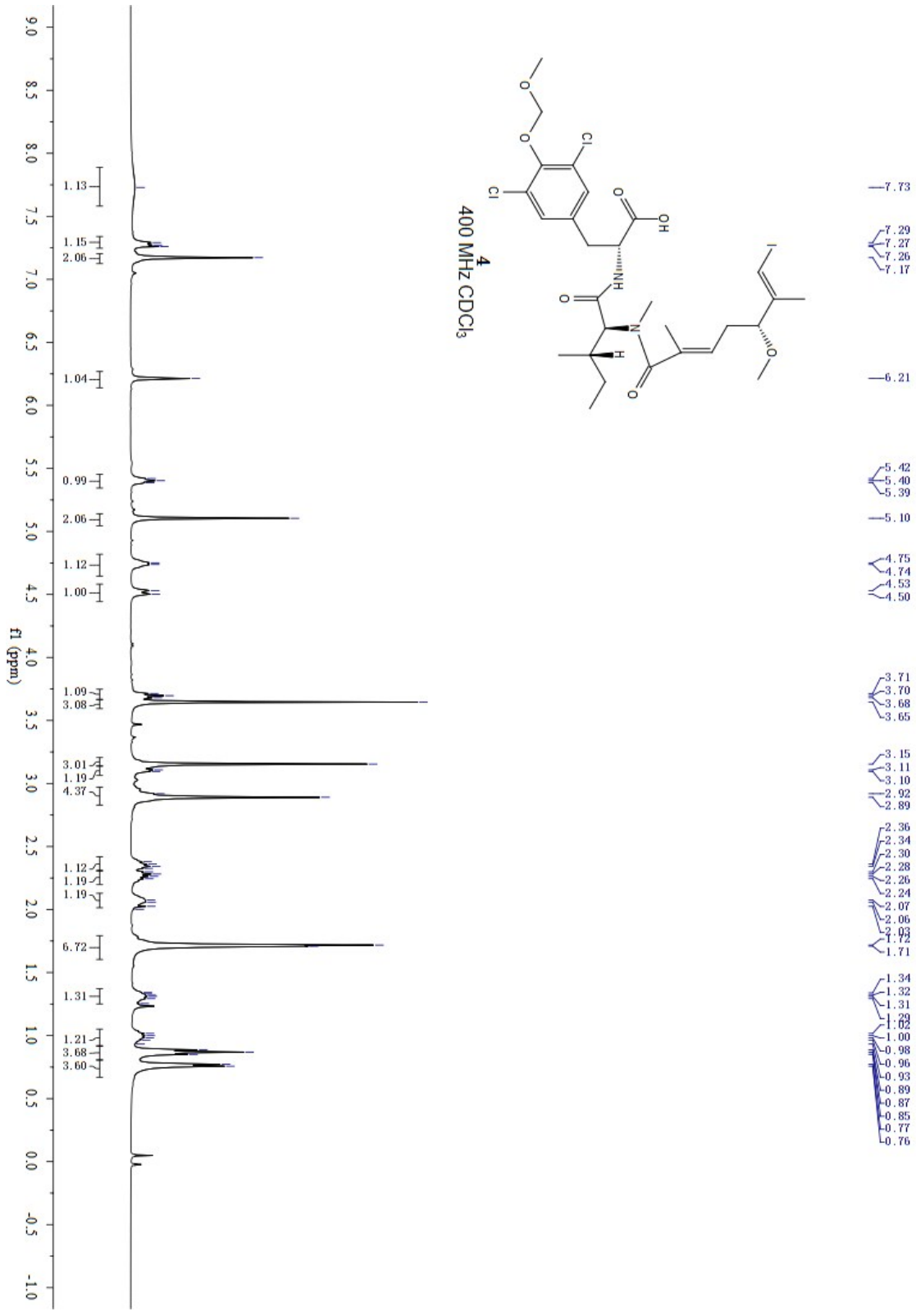

3
100 MHz CDCl3
f1 (ppm)
172.67
148.69
138.68
129.76
127.99
127.89
127.86
80.22
77.48
77.16
76.84
75.12
65.17
46.28
29.10
27.96
27.34
25.52
16.29
13.79
9.44
7.11
6.67

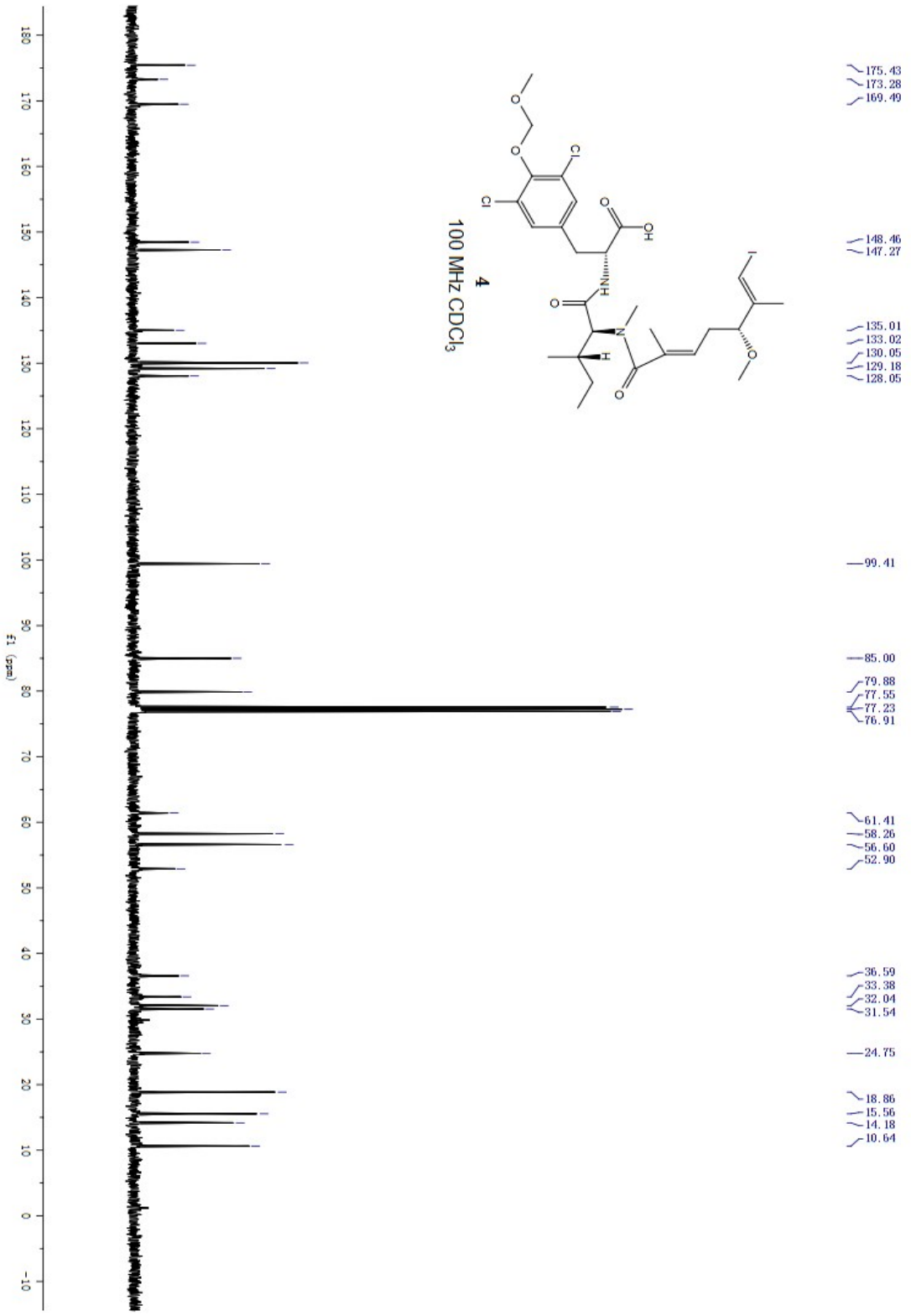

3
400 MHz C6D6
f1 (ppm)
7.33
7.31
7.18
7.16
7.14
7.08
7.06
7.05
6.09
6.05
6.00
5.94
5.94
5.93
5.91
5.90
5.89
5.88
5.88
3.44
3.40
2.89
2.87
2.86
2.84
2.82
2.80
1.99
1.57
1.55
1.53
1.51
1.50
1.49
1.37
1.35
1.33
1.32
1.31
1.19
1.16
1.14
0.95
0.94
0.61
0.59
0.57
0.54
0.52
0.50
0.48
2.16
6.18
1.12
0.07
1.02
1.97
0.14
1.00
0.07
1.15
6.65
6.84
3.25
3.27
3.46
24.71
0.70
0.50
6.68
1.00
0.07
3.44
3.40

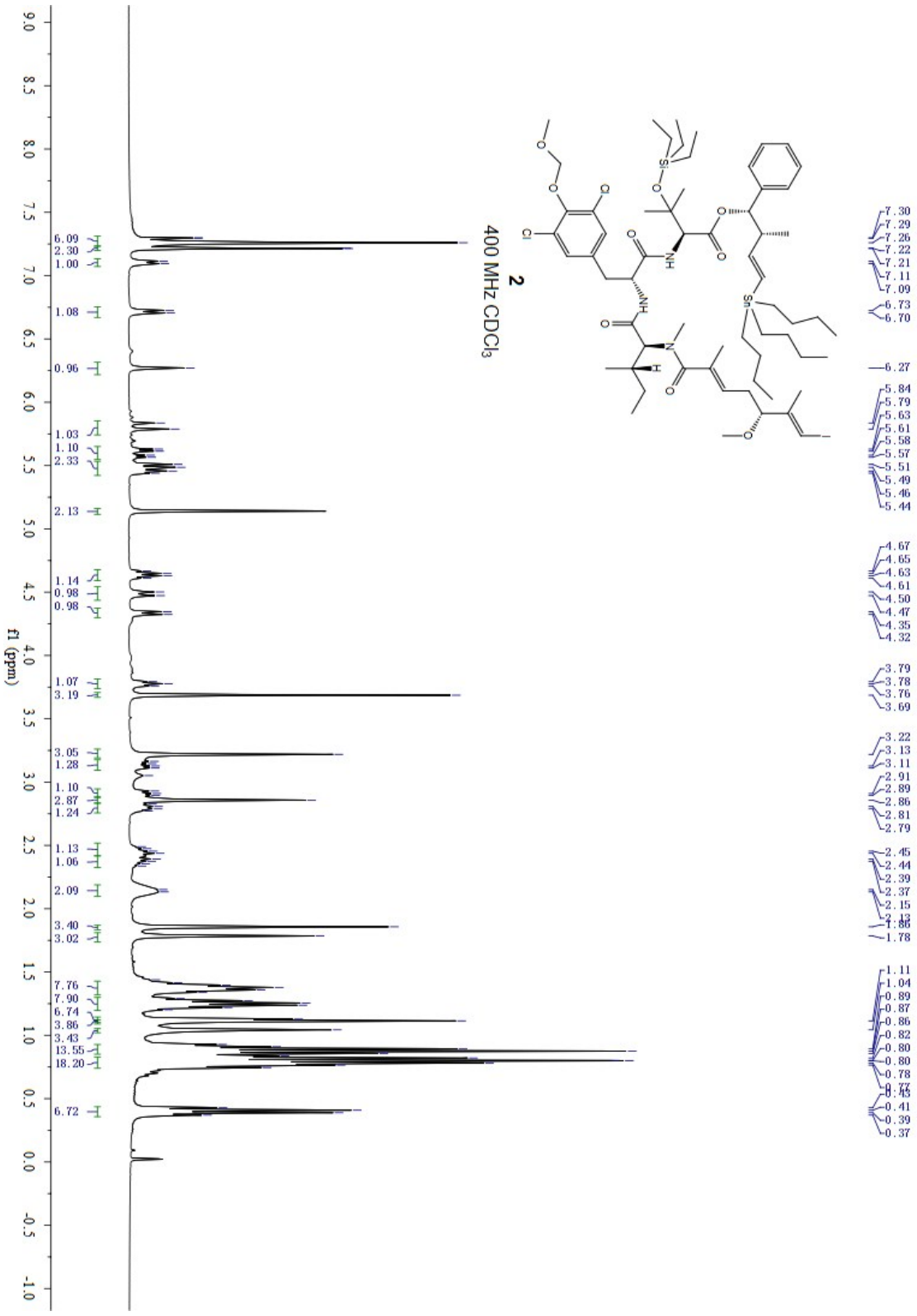

3
100 MHz C6D6
f1 (ppm)
172.93
149.71
139.46
129.55
128.30
128.18
127.94
127.82
79.67
75.68
65.45
47.00
29.51
27.71
27.56
26.29
16.32
13.98
9.73
7.34
7.00

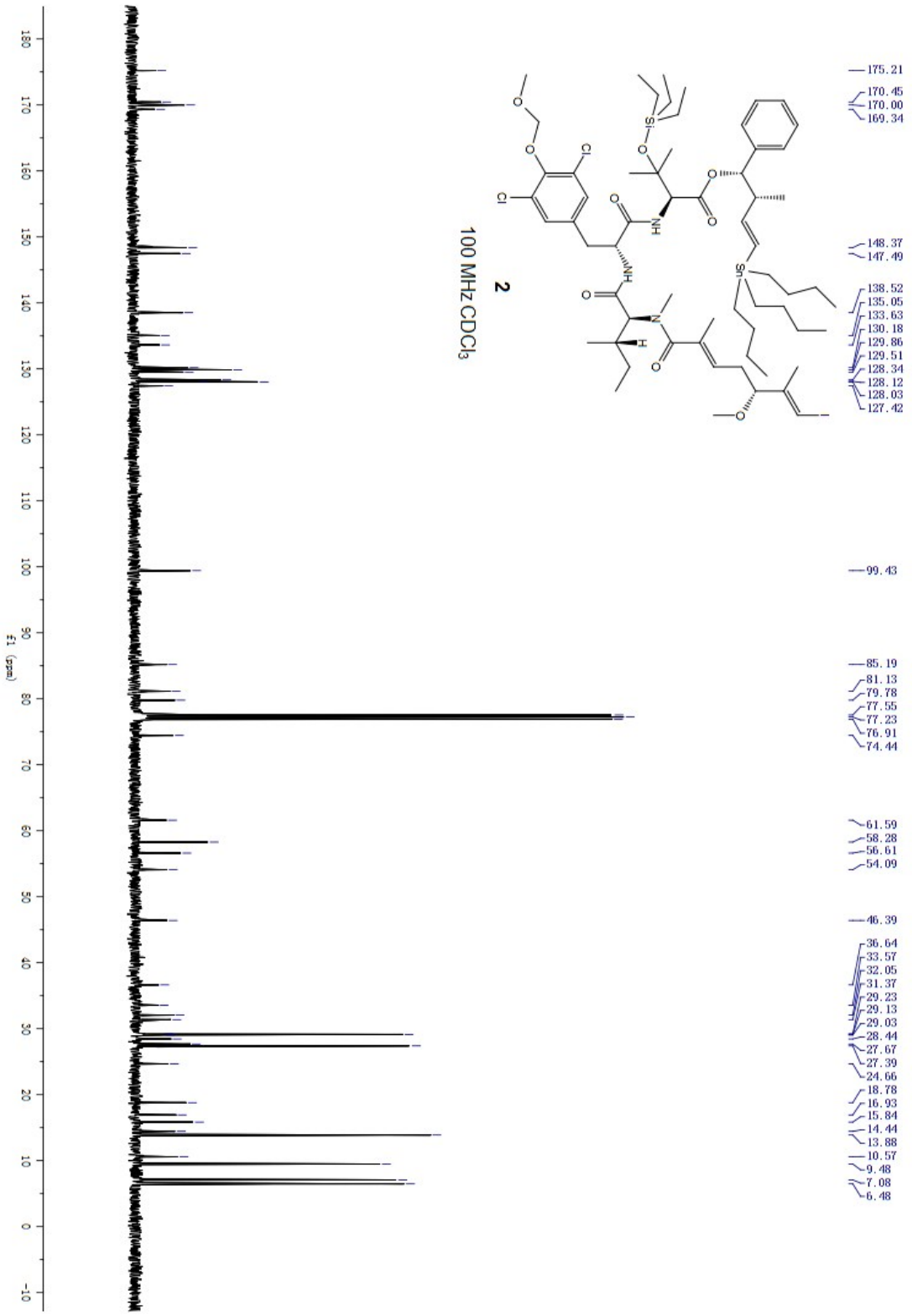

18
400 MHz CDCl3
f1 (ppm)

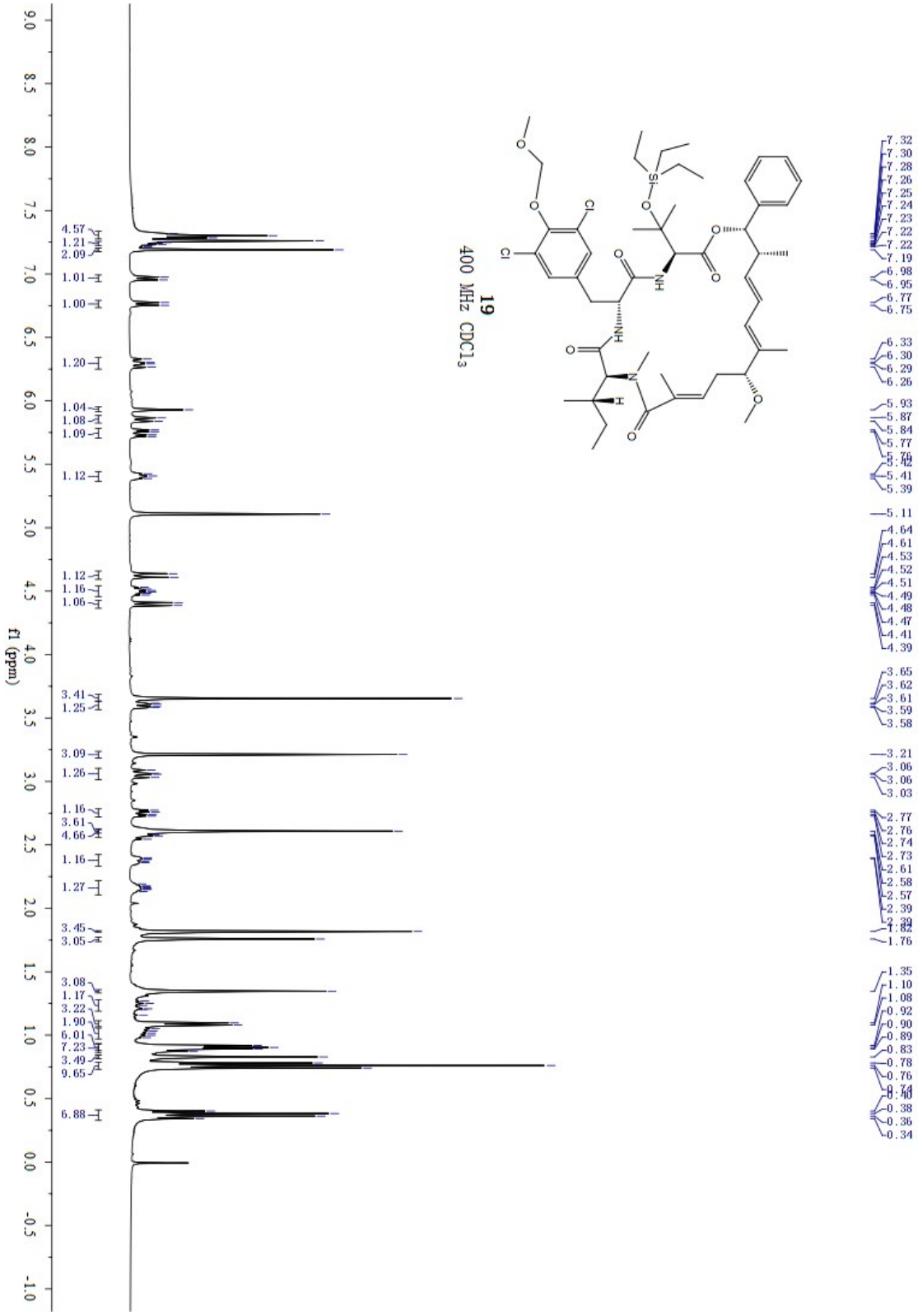

18
100 MHz CDCl3
175.33
171.27
170.10
148.66
147.39
134.30
133.48
129.72
129.40
127.64
99.41
85.07
79.65
60.92
58.23
56.56
52.72
52.55
36.74
33.13
32.06
30.90
24.55
18.80
15.73
14.33
10.53
f1 (ppm)

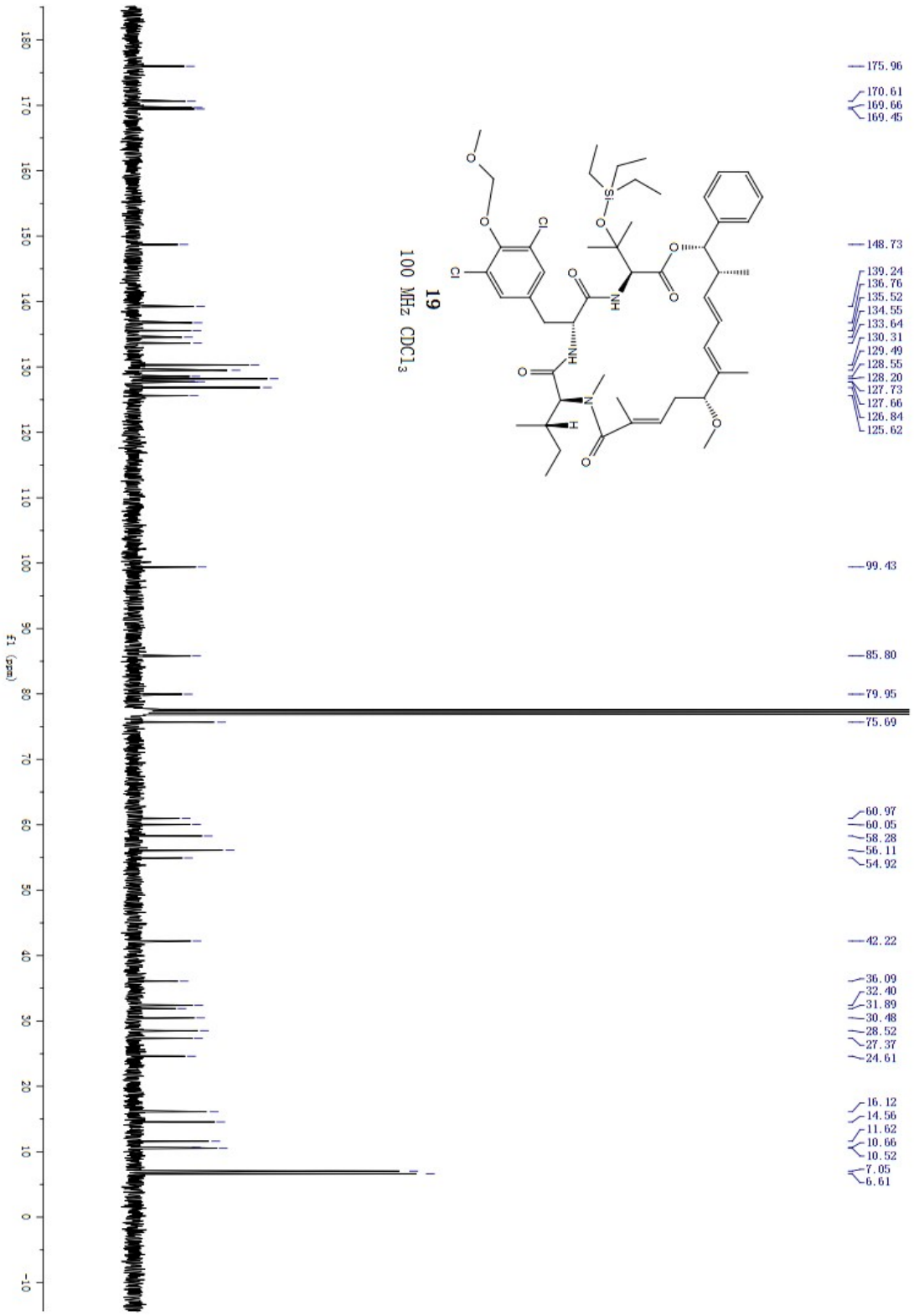

4
400 MHz CDCl3
f1 (ppm)
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
1.13
1.15
2.06
1.04
0.99
2.06
1.12
1.00
1.09
3.08
3.01
1.19
4.37
1.12
1.19
1.19
6.72
1.31
1.21
3.68
3.60
7.73
7.29
7.27
7.26
7.17
6.21
5.42
5.40
5.39
5.10
4.75
4.74
4.53
4.50
3.71
3.70
3.68
3.65
3.15
3.11
3.10
2.92
2.89
2.36
2.34
2.30
2.28
2.26
2.24
2.07
2.06
2.03
1.72
1.71
1.34
1.32
1.31
1.29
1.02
1.00
0.98
0.96
0.93
0.89
0.87
0.85
0.77
0.76

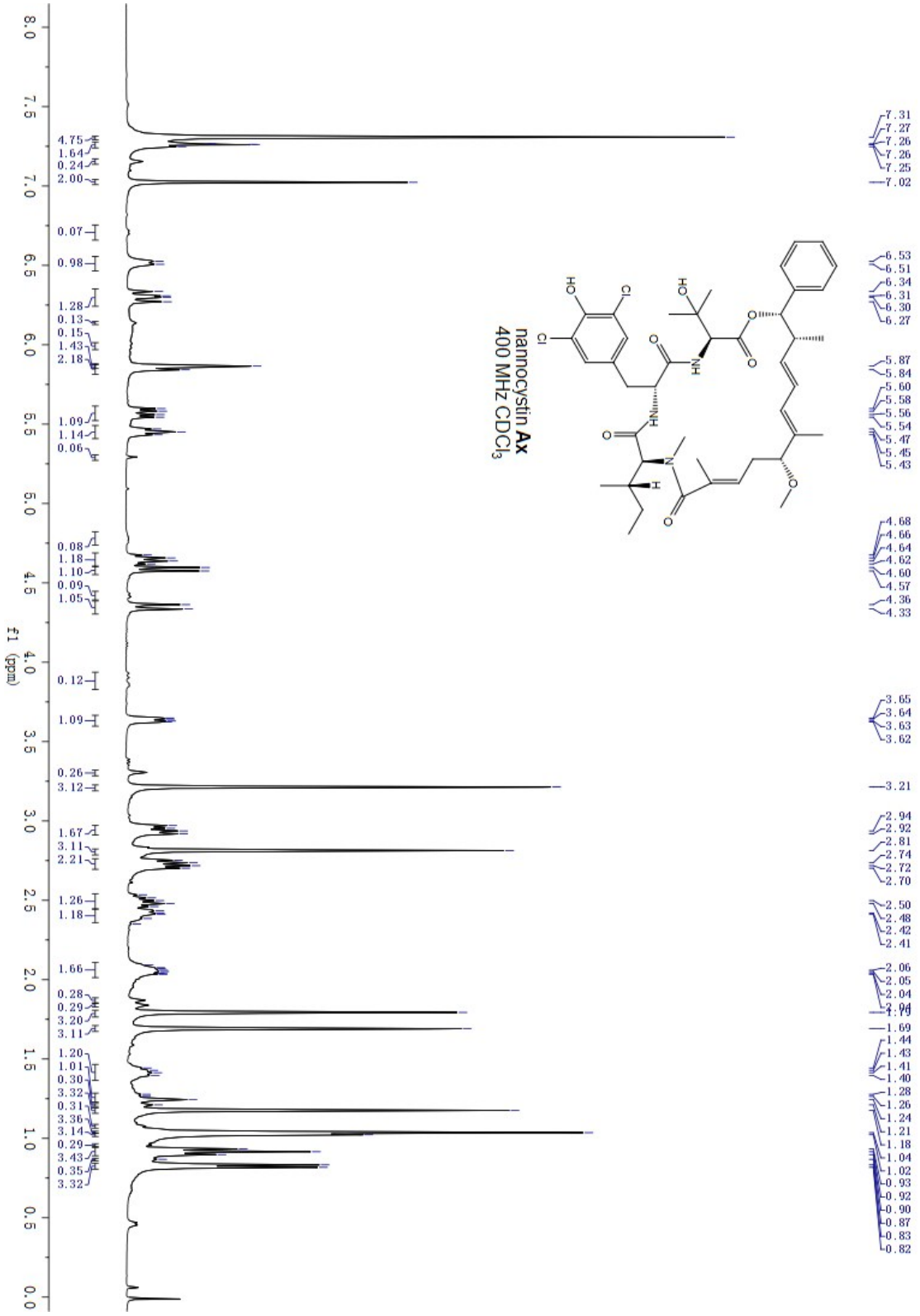

4
100 MHz CDCl3
f1 (ppm)
175.43
173.28
169.49
148.46
147.27
135.01
133.02
130.05
129.18
128.05
99.41
85.00
79.88
77.55
77.23
76.91
61.41
58.26
56.60
52.90
36.59
33.38
32.04
31.54
24.75
18.86
15.56
14.18
10.64

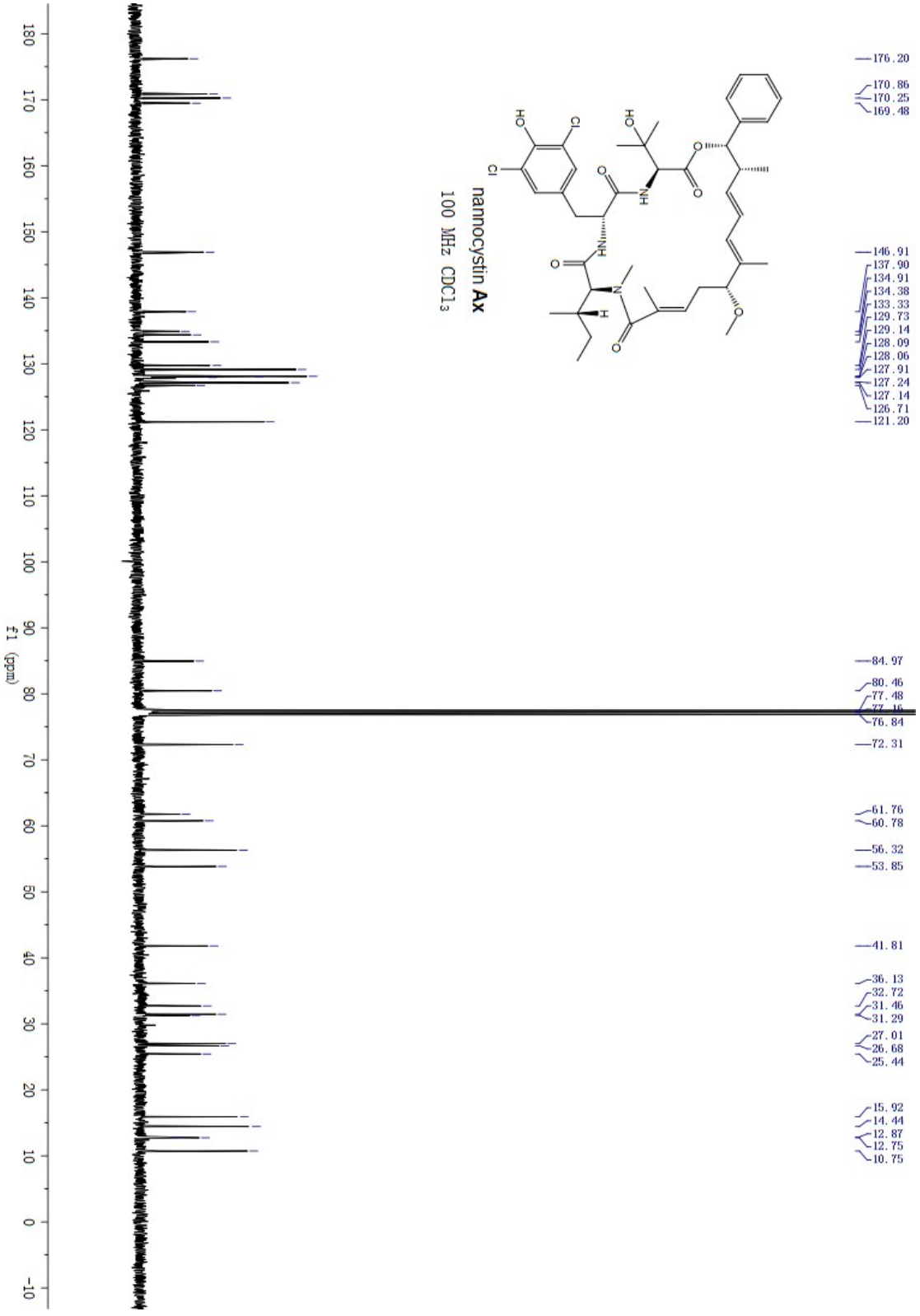

2
400 MHz CDCl3
f1 (ppm)

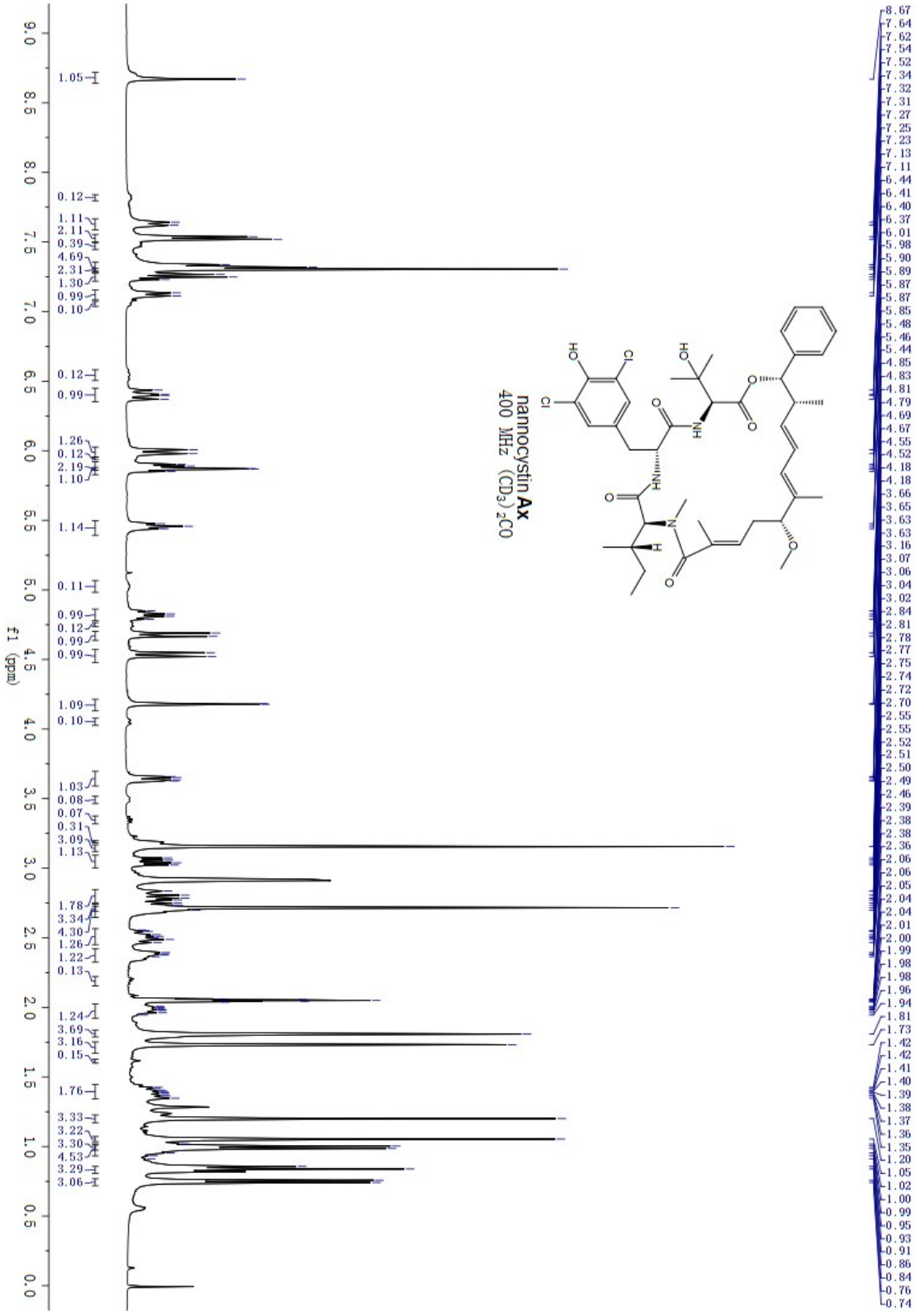

2
100 MHz CDCl3
175.21
170.45
170.00
169.34
148.37
147.49
138.52
135.05
133.63
130.18
129.86
129.51
128.34
128.12
128.03
127.42
99.43
85.19
81.13
79.78
77.55
77.23
76.91
74.44
61.59
58.28
56.61
54.09
46.39
36.64
33.57
32.05
31.37
29.23
29.13
29.03
28.44
27.67
27.39
24.66
18.78
16.93
15.84
14.44
13.88
10.57
9.48
7.08
6.48
f1 (ppm)

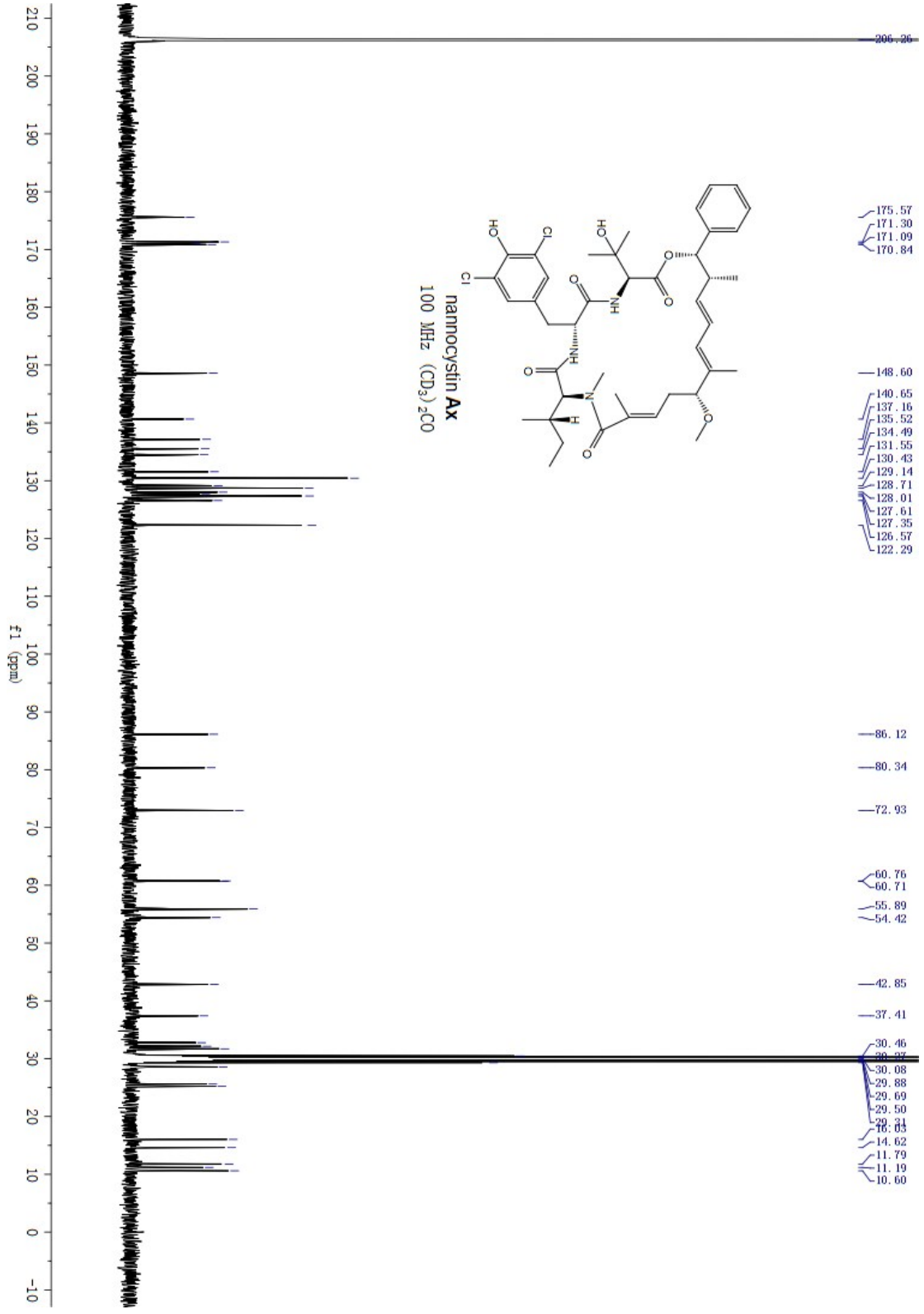

19
400 MHz CDCl3
f1 (ppm)

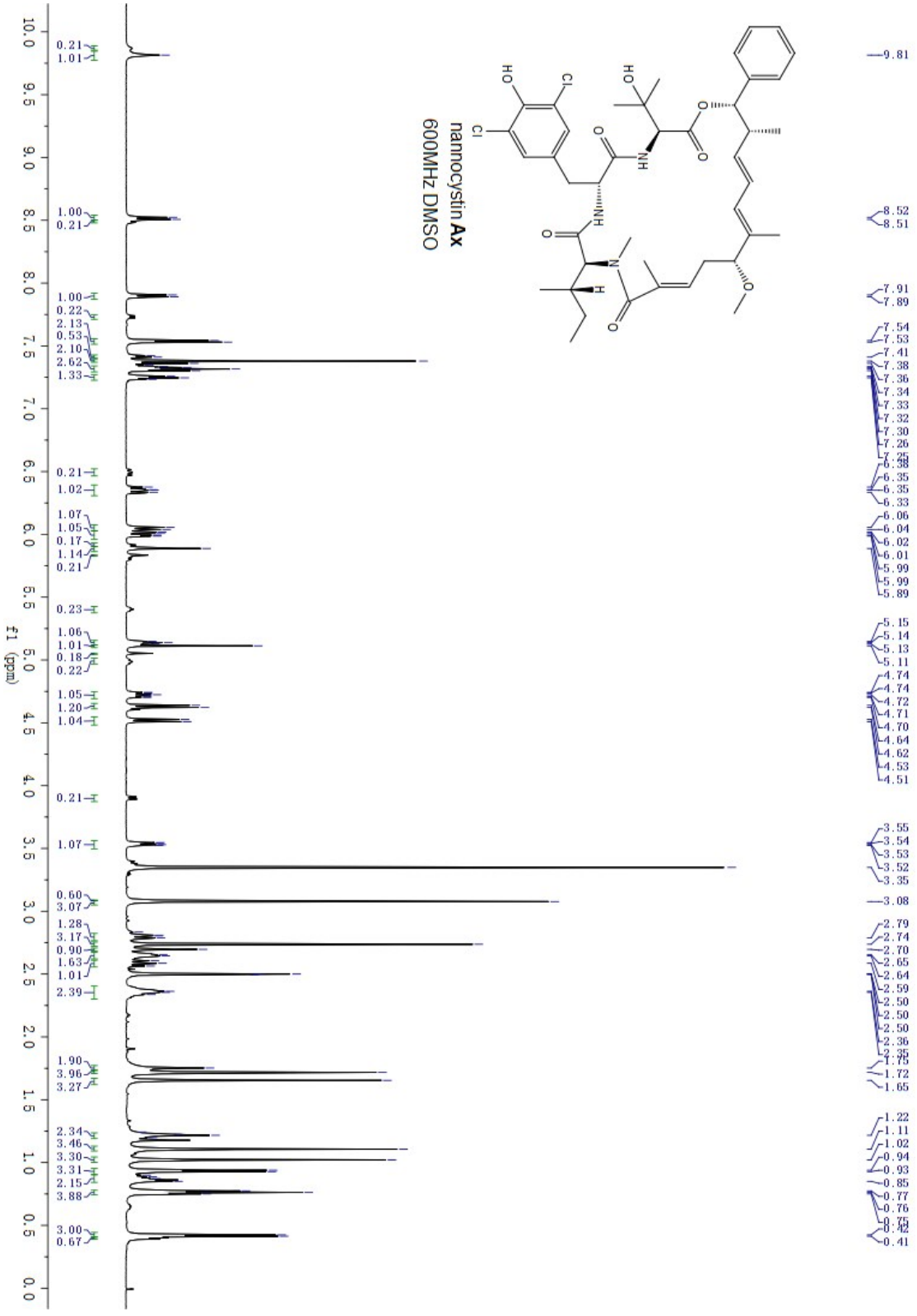

19
100 MHz CDCl3
175.96
170.61
169.66
169.45
148.73
139.24
136.76
135.52
134.55
133.64
130.31
129.49
128.55
128.20
127.73
127.66
126.84
125.62
99.43
85.80
79.95
75.69
60.97
60.05
58.28
56.11
54.92
42.22
36.09
32.40
31.89
30.48
28.52
27.37
24.61
16.12
14.56
11.62
10.66
10.52
7.05
6.61
f1 (ppm)

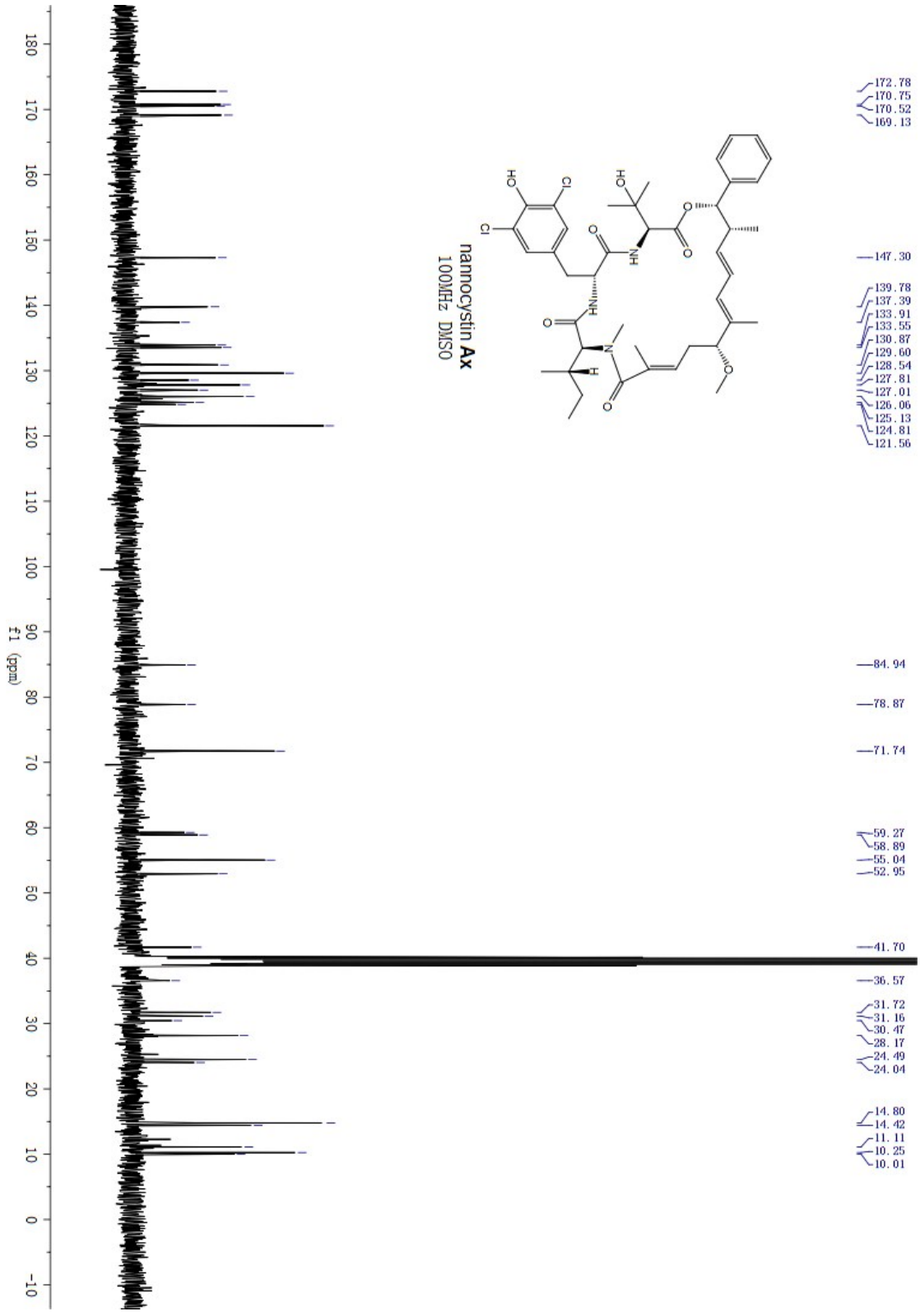

nannocystin Ax
400 MHz CDCl3
f1 (ppm)

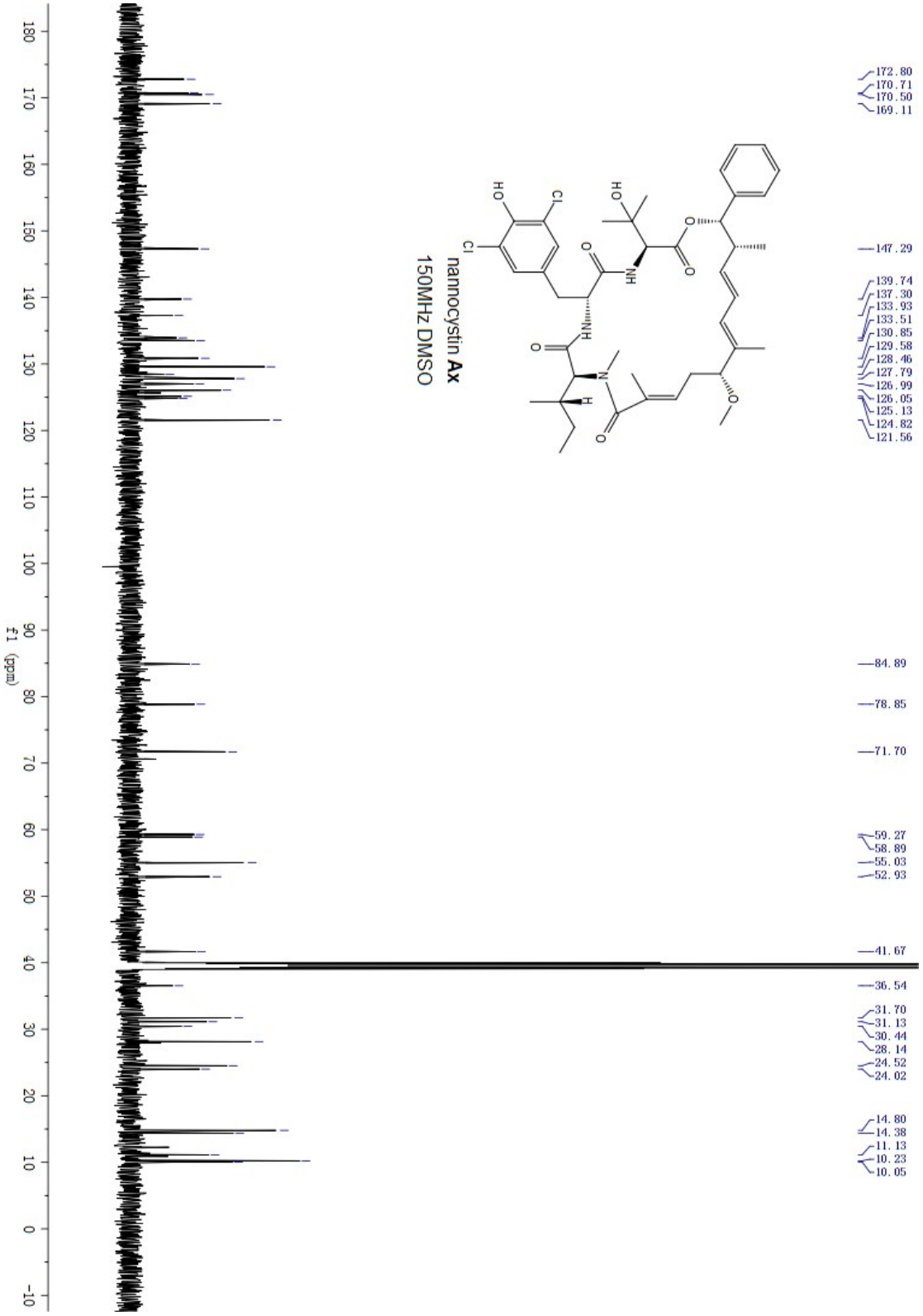

nannocystin Ax
100 MHz CDCl3
f1 (ppm)
176.20
170.86
170.25
169.48
146.91
137.90
134.91
134.38
133.33
129.73
129.14
128.09
128.06
127.91
127.24
127.14
126.71
121.20
84.97
80.46
77.48
76.84
72.31
61.76
60.78
56.32
53.85
41.81
36.13
32.72
31.46
31.29
27.01
26.68
25.44
15.92
14.44
12.87
12.75
10.75


nannocystin Ax
400 MHz (CD3)2CO
f1 (ppm)


nannocystin Ax
100 MHz (CD3)2CO
f1 (ppm)
206.26
175.57
171.30
171.09
170.84
148.60
140.65
137.16
135.52
134.49
131.55
130.43
129.14
128.71
128.01
127.61
127.35
126.57
122.29
86.12
80.34
72.93
60.76
60.71
55.89
54.42
42.85
37.41
30.46
30.08
29.88
29.69
29.50
29.31
16.03
14.62
11.79
11.19
10.60


nannocystin Ax
600MHz DMSO
f1 (ppm)


nannocystin Ax
100MHz DMSO
f1 (ppm)
172.78
170.75
170.52
169.13
147.30
139.78
137.39
133.91
133.55
130.87
129.60
128.54
127.81
127.01
126.06
125.13
124.81
121.56
84.94
78.87
71.74
59.27
58.89
55.04
52.95
41.70
36.57
31.72
31.16
30.47
28.17
24.49
24.04
14.80
14.42
11.11
10.25
10.01


nannocystin Ax
150MHz DMSO
f1 (ppm)
172.80
170.71
170.50
169.11
147.29
139.74
137.30
133.93
133.51
130.85
129.58
128.46
127.79
126.99
126.05
125.13
124.82
121.56
84.89
78.85
71.70
59.27
58.89
55.03
52.93
41.67
36.54
31.70
31.13
30.44
28.14
24.52
24.02
14.80
14.38
11.13
10.23
10.05

7. References

(1) S. Shirakawa, M. Komiyama, T. Nakamura, M. Okada, A. Nakazaki, S. Hosokawa and S. Kobayashi, *J. Am. Chem. Soc.,* 2004, **126**, 13604.

(2) S. Kajiyama, K. Irie, T. Kido, K. Koshimizu, H. Hayashi and M. Arai, *Tetrahedron*., 1991, **47**, 5453.

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