Supplementary Material

Synthesis and anti-tumor activity evaluation of salinomycin C20-*O*-alkyl/benzyl oximes derivatives

Bo Li[a #], Jun Wu[b,d,e#], Lei Tang[c#], Xu Lian[a], Zhongwen Li[b,d,e], Wenfang Duan[c], Tong Qin[a], Xintong Zhao[a], Yuhua Hu[a], Chi Zhang[a], Tianlei Li[a], Jie Hao[b,d,e], Wenxuan Zhang[a], Jihong Zhang[c]*, Song Wu[a]**

a. State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing 100050, China

b. State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China

c. Medical School of Kunming University of Science and Technology, Kunming, 650031, China

d. Institute for Stem Cell and Regeneration, Chinese Academy of Sciences, Beijing 100101, China

e. National Stem Cell Resource Center, Chinese Academy of Sciences, Beijing 100101, China

#These authors contributed equally to this work.

*Corresponding Author: wxzhang@imm.ac.cn, Zhjihong2000@kust.edu.cn, ws@imm.ac.cn

Table of Content

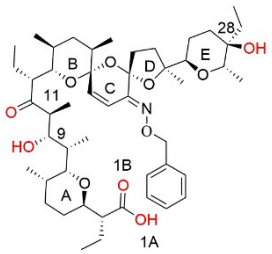
Table S1: Crystal data and structure refinement for Compound 10.

Identification code	Compound 10 (LB0302)
Empirical formula	$C_{51}H_{78}N_2O_{11}$
Formula weight	895.15
Temperature/K	293(2)
Crystal system	orthorhombic
Space group	$P2_12_12_1$
a/Å	10.28331(11)
b/Å	15.0300(2)
c/Å	33.3759(5)
α/°	90
β/°	90
γ/°	90
Volume/Å^3	5158.52(11)
Z	4
ρ_{calc}g/cm^3	1.153
μ/mm^{-1}	0.646
F(000)	1944.0
Crystal size/mm^3	0.3 × 0.26 × 0.24
Radiation	CuKα (λ = 1.54184)
2Θ range for data collection/°	6.45 to 133.65
Index ranges	$-12 \leq h \leq 10$, $-17 \leq k \leq 17$, $-39 \leq l \leq 37$
Reflections collected	42181
Independent reflections	9137 [R_{int} = 0.0306, R_{sigma} = 0.0219]
Data/restraints/parameters	9137/0/592
Goodness-of-fit on F^2	1.032
Final R indexes [I>=2σ (I)]	R_1 = 0.0327, wR_2 = 0.0834
Final R indexes [all data]	R_1 = 0.0370, wR_2 = 0.0873
Largest diff. peak/hole / e Å^{-3}	0.13/-0.18
Flack parameter	-0.07(5)

Procedure for crystallization of compound 10:

Purified compound **10** (20 mg) was dissolved in Acetonitrile (1 mL, HPLC grade), and then the solution was filtered through a 0.45 μm nylon filter into a small sample vial. The vial was covered with Parafilm and punctured at the top. Then the vial was placed on a stand shelf.

Table S2: The chemical shift (ppm) of proton and carbon signals of compound 10 in CH_3OD



10

Position	1H/13C (ppm)
2	2.94/49.8
3	3.97/76.41
7	3.67/72.92
9	4.07/70.58
10	3.12/48.29
12	2.73/57.76
13	3.66/80.3
25	3.35/73.87
29	3.76/77.25

Table S3: Cytotoxicity on neuroblastoma cell (SH-SY5Y) and tumor-targeting selectivity (SI).

Compound	**IC_{50}**	**SI**			**IC_{50}**	**SI**		
	SH-SY5Y	**HT-29**	**HGC-27**	**MDA-MB-231**	**Compound**	**SH-SY5Y**	**HT-29**	**HGC-27**	**MDA-MB-231**
1	~1	0.36	0.41	0.14	**11**[a]	0.896	13.8	29.9	2.2
2	>1	>2.7	>1.8	>1.1	**12**	>1	>28.6	>41.7	>3.26
3	>1	>20.0	>33.3	>11.1	**13**	>1	>33.3	>11.5	>14.9
4	>1	>33.3	>11.5	>15.2	**14**	>1	>27.8	>37.0	>10.3
5	>1	>33.3	>11.6	>10.9	**15**[a]	0.277	7.5	12.6	1.5
6	>1	>33.3	>23.2	>27.0	**16**	>1	>33.3	>10.6	>25
7	>1	>33.3	>10.8	>11.5	**17**	>1	>19.6	>34.5	>7.0
8	>1	>33.3	>10.9	>11.8	**18**[a]	0.654	24.2	65.4	6
9	nd	-	-	-	**19**	>1	>21.7	>35.7	>5.6
10	>1	>33.3	>38.5	>8.2					

SI (selectivity index) was calculated using the formula: SI = IC_{50} for neuroblastoma cell line SH-SY5Y/ IC_{50} for respective cancer cell lines (HT-29, HGC- 27 and MDA-MB-231). nd, not determined; [a] Compounds showed toxicity in neuroblastoma cell line SH-SY5Y with IC_{50} < 1 µM.

1. Chemistry experiments

1.1 General experimental information

All reactions were performed in glassware containing a Tefloncoated stir bar. Solvents and chemical reagents were obtained from commercial sources and used without further purifications. ^{1}H and ^{13}C NMR spectra were recorded on Bruker 400, 500 or 600 MHz NMR spectrometer using $CDCl_3$ or CD_3OD as the solvent. Chemical shifts (δ) were reported in ppm downfield from an internal TMS standard. Low or High-resolution mass spectra were obtained in the ESI mode. Flash column chromatography on silica gel (200-300 mesh) was used for the routine purification of reaction products. The column output was monitored by TLC on silica gel (100-200 mesh) precoated on glass plates(15×50 mm), and spots were visualized by 5% vanillin sulfuric acid/ethanol solution.

1.2 O-substituted hydroxylamine hydrochloride

1.2.1 *O*-alkyl hydroxylamine hydrochloride

In this work, several *O*-alkyl hydroxylamine hydrochloride were purchased from Innochem® . The chemical name and corresponding CAS number are listed below:

Methoxyamine hydrochloride, CAS: 593-56-6

O-Ethylhydroxylamine hydrochloride, CAS: 3332-29-4

O-(tert-Butyl)hydroxylamine hydrochloride, CAS: 39684-28-1

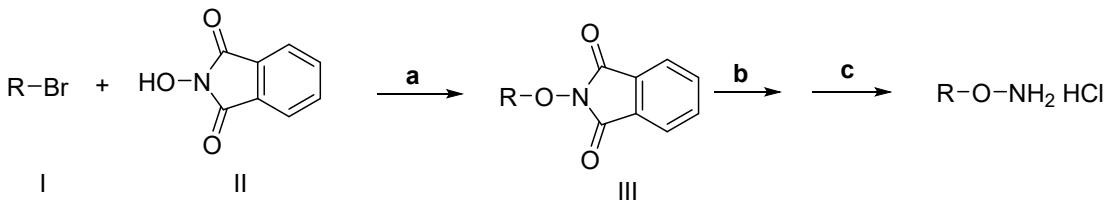
O-Allylhydroxylamine Hydrochloride, CAS: 38945-21-0

(E)-*O*-(3-Chloroallyl)hydroxylamine Hydrochloride, CAS: 96992-71-1

O-Prop-2-ynyl-hydroxylamine hydrochloride, CAS: 21663-79-6

O-(Carboxymethyl)hydroxylamine hemihydrochloride, CAS: 2921-14-4

1.2.2 *O*-benzyl hydroxylamine hydrochloride



O-benzyl hydroxylamine hydrochloride intermediates used in this study were all synthesized according to the protocol published by Fan et al.[1]

To a solution of *N*-hydroxyphthalimide (NOP, 40 mmol, 1.0 equiv) in DMF were added potassium carbonate (80 mmol, 2.0 equiv) and substituted benzyl bromide (40 mmol, 1.0 equiv). The mixture was stirred at room temperature for 6 h. Subsequently, the mixture was pour into ice-cooled water and the precipitate was filtered and washed by ice-cooled water twice. The solid (**III**) was dried and direct used for next step without further purification.

A mixture of intermediate **III** (20 mmol, 1.0 equiv) and hydrozine hydrate (50 mmol, 2.5 equiv) in the mixed solution of dichloromethane (120 mL) and methanol (12 mL) was stirred at room temperature for 2 h. After filtration, the filtrate was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was precipitated in HCl solution (2 M in ether) and then filtered to give corresponding *O*-benzyl hydroxylamine hydrochloride as white solid and used direct in next step without further purification.

1.3 General procedure of synthesis of C20 O-alkyl/benzyl oxime salinomycin

To the solution of Salinomycin (500 mg, 0.7 mmol) in dichloromethane (5 mL) was added Dess-Martin periodinane (DMP, 1.1 g, 2.7 mmol). The reaction was allowed to stirred at room temperature for 30 min. The reaction mixture was diluted by ethyl acetate (20 mL) and washed with 0.1 M NaOH twice, 0.1 M HCl twice, water once and brine. The organic solution was dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified through flash chromatography (eluent: petroleum ether/ethyl acetate= 5/1) to give C20-oxo salinomycin (**2**) as white solid (300 mg, 60%).$[\alpha]_D^{20}$=5 (c = 0.1, CH_3OH), ^{1}H NMR (400 MHz, CDCl3) δ 7.15 (d, *J* = 10.8 Hz, 1H), 6.22 (d, *J* = 10.8 Hz, 1H), 4.14 (d, *J* = 6.5 Hz, 1H), 4.07 – 3.91 (m, 2H), 3.79 (d, *J* = 10.1 Hz, 1H), 3.59 (d, *J* = 9.9 Hz, 1H), 3.49 (d, *J* = 5.2 Hz, 1H), 2.89 – 2.78 (m, 1H), 2.74 –2.63 (m, 1H), 2.62 –2.48 (m, 2H), 2.06 – 0.66 (m, 55H). ^{13}C NMR (101 MHz, CDCl3) δ 213.66, 190.47, 177.79, 143.62, 126.39, 105.84, 97.73, 91.18, 77.24, 75.43, 74.98, 74.85, 74.76, 72.43, 71.54, 68.10, 55.10, 49.54, 49.09, 40.13, 38.91, 36.41, 35.02, 34.12, 32.78, 31.26, 30.43, 29.73, 27.98, 26.32, 24.27, 22.68, 21.75, 19.71, 17.96, 16.10, 15.97, 13.92, 12.99, 12.95, 12.09, 10.77, 6.69.

To the solution of C20-oxo salinomycin (100 mg, 0.134 mmol, 1.0 equiv) in 2 mL methanol were added sodium methoxide (14.5 mg, 0.268 mmol, 2.0 equiv) and *O*-substituted hydroxylamine hydrochloride (0.268 mmol, 2.0 equiv). After stirring at room temperature for 12 h, the reaction solution was poured into 0.1 M HCl solution (for compound **4**, **5**, **11**, **17** and **19**, the reaction mixture

[1]Fan, Y. L., Wu, J. B., Ke, X., & Huang, Z. P. (2018). Design, synthesis and evaluation of oxime-functionalized nitrofuranylamides as novel antitubercular agents. *Bioorganic & medicinal chemistry letters*, *28*(18), 3064-3066.

was poured into water) and filtered to obtain a white precipitate. After purification by column chromatography (mobile phase: petroleum ether: ethyl acetate = 1:1), a white foamy solid was obtained.

Methyl derivative 3

Yield 46%, white foamed solid, HRMS-ESI (*m/z*): $[M+Na]^+$ Calcd for $C_{43}H_{71}NO_{11}Na$ 800.4925; Found 800.4940, ^1H NMR (400 MHz, $CDCl_3$) δ 6.89 (d, *J* = 10.9 Hz, 1H), 6.50 (d, *J* = 10.9 Hz, 1H), 4.13 – 4.10 (m, 1H), 4.02 (dd, *J* = 11.0, 5.7 Hz, 1H), 3.86 (m, 4H), 3.82 (d, *J* = 18.2 Hz, 1H), 3.67 (dd, *J* = 9.9, 2.2 Hz, 1H), 3.57 – 3.52 (m, 1H), 2.92 (td, *J* = 11.1, 3.9 Hz, 1H), 2.73 (ddd, *J* = 10.8, 5.9, 3.2 Hz, 2H), 2.61 (dd, *J* = 10.9, 2.6 Hz, 1H), 2.09 – 0.65 (m, 55H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 213.62, 177.40, 148.48, 130.86, 116.24, 105.08, 98.39, 89.76, 75.29, 75.08, 74.98, 74.73, 71.92, 71.55, 68.13, 62.00, 55.47, 49.39, 49.14, 40.30, 38.84, 36.31, 35.93, 34.19, 32.81, 30.27, 28.03, 26.41, 24.95, 22.60, 21.78, 19.89, 17.94, 16.08, 14.08, 13.18, 12.98, 11.93, 10.96, 6.84, 6.68.

Ethyl derivative 4 (Sodium salt)

Yield 51%, white foamed solid, HRMS-ESI (*m/z*): $[M+Na]^+$ Calcd for $C_{44}H_{73}NO_{11}Na$ 814.5076; Found 814.5080, ^1H NMR (500 MHz, $CDCl_3$) δ 6.90 (d, *J* = 11.0 Hz, 1H), 6.45 (d, *J* = 10.8 Hz, 1H), 4.17 – 4.00 (m, 3H), 3.96 (dd, *J* = 13.1, 6.1 Hz, 2H), 3.84 (d, *J* = 10.1 Hz, 1H), 3.62 (d, *J* = 9.8 Hz, 1H), 3.49 (d, *J* = 8.8 Hz, 1H), 2.87 (t, *J* = 11.3 Hz, 1H), 2.72 (dt, *J* = 27.6, 10.0 Hz, 2H), 2.57 (d, *J* = 10.6 Hz, 1H), 2.20 – 0.40 (m, 55H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 216.71, 183.76, 145.97, 129.94, 116.83, 105.12, 98.75, 88.68, 76.07, 75.94, 75.20, 74.29, 71.35, 70.22, 69.66, 67.03, 55.20, 51.18, 50.43, 40.24, 38.50, 35.86, 34.90, 33.10, 32.33, 32.31, 29.57, 28.03, 27.53, 26.97, 23.63, 20.40, 20.13, 17.44, 16.01, 15.95, 14.67, 14.57, 13.12, 12.40, 11.97, 10.78, 6.88, 6.43.

Tertiary butyl derivative 5 (Sodium salt)

Yield 47%, white foamed solid, HRMS-ESI (*m/z*): $[M+Na]^+$ Calcd for $C_{46}H_{77}NO_{11}Na$ 842.5394; Found 842.5391. ^1H NMR (500 MHz, $CDCl_3$) δ 7.00 (d, *J* = 10.8 Hz, 1H), 6.47 (d, *J* = 11.0 Hz, 1H), 4.28 (dd, *J* = 15.7, 8.6 Hz, 2H), 3.95 (dd, *J* = 11.1, 4.5 Hz, 1H), 3.79 (d, *J* = 9.9 Hz, 1H), 3.66 (d, *J* = 10.2 Hz, 1H), 3.45 – 3.32 (m, 1H), 3.01 – 2.87 (m, 1H), 2.84 (td, *J* = 11.0, 3.4 Hz, 1H), 2.71 (ddd, *J* = 28.4, 10.6, 4.8 Hz, 1H), 2.22 – 0.42 (m, 66H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 216.64, 183.65, 144.50 , 129.04 , 117.05 , 105.65 , 98.86 , 88.72 , 77.24 , 76.09 , 76.00 , 75.11 , 74.20 , 71.33 , 69.48 , 66.93 , 55.20 , 51.15 , 50.66 , 40.39 , 38.59 , 35.90 , 34.56 , 33.13 , 32.39 , 32.30 , 29.73 , 29.48 , 28.03 , 27.64 , 27.58 , 27.00 , 23.70 , 22.73 , 20.46 , 20.09 , 17.49 , 16.14 , 16.10 , 14.69 , 13.19 , 12.60 , 11.92 , 10.77 , 6.87 , 6.44 .

Allylic derivative 6

Yield 58%, white foamed solid, $[\alpha]_D^{20}$=18 (c = 0.1, CH_3OH), HRMS-ESI (*m/z*): $[M+Na]^+$ Calcd for$C_{45}H_{73}NO_{11}Na$ 826.5081; Found 826.5079, ^1H NMR (500 MHz, $CDCl_3$) δ 6.92 (d, *J* = 10.9 Hz, 1H), 6.47 (d, *J* = 10.9 Hz, 1H), 5.92 (ddt, *J* = 16.3, 10.6, 5.6 Hz, 1H), 5.21 (d, *J* = 17.3 Hz, 2H), 4.53 (t, *J* = 5.5 Hz, 3H), 4.08 (d, *J* = 9.9 Hz, 1H), 3.96 (t, *J* = 7.7 Hz, 2H), 3.84 (d, *J* = 10.2 Hz, 1H), 3.62 (d, *J* = 10.1 Hz, 1H), 3.59 – 3.41 (m, 2H), 2.87 (td, *J* = 10.9, 3.7 Hz, 1H), 2.71 (p, *J* = 9.0, 8.6 Hz, 2H), 2.58 (d, *J* = 10.5 Hz, 1H), 2.12 – 0.30 (m, 59H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 213.98 , 177.55 , 148.54 , 134.44 , 130.85 , 116.92 , 116.35 , 105.06 , 98.46 , 89.72 , 77.25 , 75.44 , 75.06 , 75.01 , 74.94 , 74.41 , 71.81 , 71.56 , 68.11 , 55.52 , 49.44 , 49.27 , 40.37 , 38.88 , 36.38 , 35.78 , 33.97 , 32.81 , 30.17 , 29.73 , 28.01 , 27.27 , 26.36 , 24.93 , 22.65 , 21.68 , 19.85 , 17.96 , 16.11 , 16.08 , 14.06 , 13.18 , 12.97 , 12.00 , 10.93 , 6.79 , 6.65 .

Chlorinated allylic derivative 7 (Sodium salt)

Yield 55%, white foamed solid, HRMS-ESI (*m/z*): $[M+Na]^+$ Calcd for $C_{45}H_{72}NO_{11}ClNa$ 860.4686; Found 860.4691, ^1H NMR (500 MHz, $CDCl_3$) δ 6.99 (d, *J* = 10.8 Hz, 1H), 6.55 (d, *J* = 11.0 Hz, 1H), 6.33 (d, *J* = 13.4 Hz, 1H), 6.22 – 6.04 (m, 1H), 4.55 (dd, *J* = 10.4, 6.7 Hz, 2H), 4.31 (d, *J* = 7.0 Hz, 1H), 4.25 (d, *J* = 10.3 Hz, 1H), 3.95

(d, *J* = 11.7 Hz, 1H), 3.77 (d, *J* = 9.8 Hz, 1H), 3.64 (d, *J* = 10.1 Hz, 1H), 3.43 (d, *J* = 11.6 Hz, 1H), 2.96 – 2.57 (m, 5H), 2.40 – 0.62 (m, 59H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 216.67, 183.76, 147.11, 130.95, 129.64, 122.01, 116.64, 104.94, 98.61, 88.75, 76.09, 76.08, 75.26, 74.24, 72.40, 71.31, 69.71, 67.05, 55.17, 51.15, 50.36, 40.10, 38.43, 35.86, 35.51, 33.16, 32.32, 32.29, 29.26, 28.05, 27.42, 27.00, 23.61, 20.39, 20.11, 17.44, 15.98, 15.89, 14.60, 13.08, 12.50, 12.00, 10.78, 6.88, 6.48.

Propinyl derivative 8

Yield 30%, white foamed solid, HRMS-ESI (*m/z*): $[M+Na]^+$ Calcd for $C_{45}H_{71}NO_{11}Na$ 824.4919; Found 824.4931, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.00 (d, *J* = 10.9 Hz, 1H), 6.57 (d, *J* = 10.9 Hz, 1H), 4.67 (dd, *J* = 2.4, 0.8 Hz, 2H), 4.35 – 4.19 (m, 2H), 3.95 (dd, *J* = 11.1, 4.7 Hz, 1H), 3.76 (dd, *J* = 10.1, 2.2 Hz, 1H), 3.66 (t, *J* = 9.1 Hz, 1H), 3.43 (dd, *J* = 12.2, 2.3 Hz, 1H), 2.92 – 2.79 (m, 2H), 2.76 – 2.64 (m, 2H), 2.56 (t, *J* = 2.4 Hz, 1H), 2.24 – 0.60 (m, 60H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 214.20, 177.71, 149.14, 131.63, 116.36, 104.83, 98.41, 89.53, 79.54, 75.90, 75.18, 74.92, 74.49, 74.10, 71.57, 71.44, 68.12, 61.72, 55.67, 49.57, 49.31, 40.32, 38.78, 36.40, 35.97, 33.73, 32.74, 29.97, 28.35, 28.00, 26.32, 25.41, 22.67, 21.39, 19.86, 17.92, 16.06, 14.12, 13.22, 12.96, 12.00, 10.93, 6.77, 6.60.

Carboxymethyl derivative 9

Yield 38%, white foamed solid, HRMS-ESI (*m/z*): $[M+Na]^+$ Calcd for $C_{44}H_{71}NO_{13}Na$ 844.4818; Found 844.4837, ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.00 (d, *J* = 10.6 Hz, 1H), 6.60 (d, *J* = 10.6 Hz, 1H), 4.68 – 4.49 (m, 3H), 4.24 (d, *J* = 10.4 Hz, 1H), 4.03 (s, 1H), 3.92 (s, 1H), 3.71 (d, *J* = 10.3 Hz, 1H), 3.57 (d, *J* = 10.0 Hz, 2H), 3.45 (d, *J* = 11.7 Hz, 1H), 3.00 – 2.43 (m, 1H), 2.41 – 0.25 (m, 60H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 214.14, 178.26, 171.28, 149.34, 132.39, 116.14, 104.92, 98.34, 89.48, 75.64, 75.17, 75.08, 73.78, 72.37, 71.62, 71.47, 68.16, 55.73, 49.78, 48.96, 40.16, 38.69, 36.40, 36.19, 33.81, 32.60, 29.97, 28.59, 28.07, 26.94, 26.32, 22.79, 20.27, 19.90, 17.95, 16.11, 15.71, 14.01, 13.38, 12.96, 11.65, 11.00, 6.80, 6.48.

Benzyl derivative 10

Yield 49%, white foamed solid, $[\alpha]_D^{20}$=19 (c = 0.1, CH_3OH), HRMS-ESI (*m/z*): $[M+Na]^+$ Calcd for $C_{49}H_{75}NO_{11}Na$ 876.5238; Found 876.5222, ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 7.29 – 7.14 (m, 5H), 6.88 (d, *J* = 10.9 Hz, 1H), 6.41 (d, *J* = 10.9 Hz, 1H), 5.01 (q, *J* = 12.8 Hz, 2H), 4.01 (d, *J* = 10.2 Hz, 1H), 3.90 (dd, *J* = 11.1, 5.7 Hz, 1H), 3.84 (q, *J* = 6.7 Hz, 1H), 3.76 (d, *J* = 10.2 Hz, 1H), 3.56 (d, *J* = 9.9 Hz, 1H), 3.31 (q, *J* = 4.3 Hz, 1H), 2.81 (td, *J* = 11.1, 3.7 Hz, 1H), 2.62 (tt, *J* = 10.6, 6.0 Hz, 2H), 2.51 (d, *J* = 9.9 Hz, 1H), 1.99 – 0.50 (m, 55H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 214.40 , 177.88 , 148.49 , 138.31 , 130.95 , 128.26 , 127.68 , 127.46 , 116.51 , 104.90 , 98.56 , 89.53 , 77.25 , 75.99 , 75. 70 , 75.06 , 74.90 , 73.95 , 71.64 , 71.59 , 68.12 , 55.58 , 49.56 , 49.39 , 40.43 , 38.90 , 36.44 , 35.46 , 33.74 , 32.83 , 29.98 , 29.76 , 29.73 , 28.00 , 26.31 , 24.88 , 22.71 , 21.33 , 19.84 , 17.98 , 16.14 , 16.10 , 14.03 , 13.20 , 12.96 , 12.04 , 10.93 , 6.77 , 6.65 .

4-Chloro-Benzyl derivative 11 (Sodium salt)

Yield 45%, white foamed solid, HRMS-ESI (*m/z*): $[M+Na]^+$ Calcd for $C_{49}H_{74}NO_{11}ClNa$ 910.4843; Found 910.4842, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (d, *J* = 8.2 Hz, 2H), 7.21 (d, *J* = 8.2 Hz, 2H), 6.93 (d, *J* = 10.8 Hz, 1H), 6.50 (d, *J* = 10.9 Hz, 1H), 5.03 (q, *J* = 13.1 Hz, 2H), 4.08 (d, *J* = 10.3 Hz, 1H), 3.98 (dd, *J* = 11.1, 5.8 Hz, 1H), 3.91 (d, *J* = 6.8 Hz, 1H), 3.82 (d, *J* = 10.2 Hz, 1H), 3.62 (d, *J* = 9.9 Hz, 1H), 3.37 (t, *J* = 5.6 Hz, 1H), 2.87 (td, *J* = 11.1, 3.7 Hz, 1H), 2.74 – 2.61 (m, 2H), 2.58 (d, *J* = 10.3 Hz, 1H), 2.07 – 0.58 (m, 55H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 216.56, 183.93, 146.95, 136.65, 133.00, 130.79, 129.34, 128.53, 116.85, 104.97, 98.75, 88.81, 76.18, 75.98, 75.31, 75.25, 74.24, 71.34, 69.68, 67.05, 55.16, 51.19, 50.36, 40.21, 38.52, 35.91, 34.85, 33.06, 32.42, 32.31, 29.15, 28.04, 27.56, 27.01, 23.72, 20.25, 20.09, 17.44, 15.96, 15.92, 14.59, 13.10, 12.56, 11.98, 10.75, 6.85, 6.58.

3-Chloro-Benzyl derivative 12

Yield 48%, white foamed solid, HRMS-ESI (*m/z*): $[M+H]^+$ Calcd for $C_{49}H_{75}NO_{11}Cl$ 888.5029; Found 888.5036, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 – 7.14 (m, 4H), 6.93 (d, *J* = 10.8 Hz, 1H), 6.50 (d, *J* = 10.9 Hz, 1H), 5.02 (q, *J* = 13.0 Hz, 2H), 4.07 (d, *J* = 10.3 Hz, 1H), 4.01 – 3.87 (m, 2H), 3.82 (d, *J* = 10.2 Hz, 1H), 3.62 (d, *J* = 9.9

Hz, 1H), 3.39 (d, *J* = 6.0 Hz, 1H), 2.87 (d, *J* = 3.5 Hz, 1H), 2.67 (ddd, *J* = 14.7, 8.8, 4.8 Hz, 2H), 2.58 (d, *J* = 10.7 Hz, 1H), 2.06 – 0.52 (m, 55H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 214.05, 177.65, 149.12, 140.42, 133.99, 131.42, 129.65, 127.74, 127.59, 125.87, 116.28, 104.93, 98.48, 89.74, 75.45, 75.11, 74.86, 74.24, 71.82, 71.57, 68.14, 55.46, 49.37, 49.30, 40.34, 38.92, 36.39, 35.59, 34.00, 32.84, 30.07, 27.99, 26.33, 24.67, 22.63, 21.44, 19.81, 17.91, 16.11, 16.03, 13.98, 13.11, 12.94, 11.95, 10.88, 6.73, 6.63.

2-Fluoro-Benzyl derivative 13

Yield 45%, white foamed solid, HRMS-ESI (*m/z*): $[M+Na]^+$ Calcd for $C_{49}H_{74}NO_{11}FNa$ 894.5144; Found 894.5140, ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 (t, *J* = 7.5 Hz, 1H), 7.27 (d, *J* = 6.9 Hz, 1H), 7.14 (t, *J* = 7.7 Hz, 1H), 7.03 (t, *J* = 9.3 Hz, 1H), 6.98 (d, *J* = 10.8 Hz, 1H), 6.54 (d, *J* = 10.8 Hz, 1H), 5.19 (s, 2H), 4.11 (d, *J* = 10.2 Hz, 1H), 4.01 (dd, *J* = 12.9, 6.3 Hz, 2H), 3.87 (d, *J* = 9.8 Hz, 1H), 3.65 (t, *J* = 10.5 Hz, 1H), 3.50 – 3.27 (m, 1H), 2.92 (td, *J* = 11.0, 3.6 Hz, 1H), 2.72 (dq, *J* = 18.8, 10.5, 8.6 Hz, 2H), 2.62 (d, *J* = 10.7 Hz, 1H), 2.11 – 0.46 (m, 60H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 213.91 , 177.62 , 149.22 , 131.28 , 130.15 , 130.11 , 129.13 , 129.05 , 125.61 , 124.02 , 123.98 , 116.14 , 115.07 , 114.85 , 105.08 , 98.50 , 90.00 , 77.24 , 75.07 , 74.96 , 74.92 , 74.60 , 72.10 , 71.56 , 69.44 , 69.40 , 68.07 , 55.30 , 49.32 , 40.33 , 38.99 , 36.39 , 35.50 , 34.33 , 32.89 , 30.17 , 28.00 , 26.35 , 24.53 , 22.65 , 21.61 , 19.80 , 19.65 , 17.98 , 16.11 , 13.96 , 13.12 , 12.94 , 11.95 , 10.88 , 6.77 , 6.72 .

4-Methoxyl Benzyl derivative 14

Yield 42%, white foamed solid, HRMS-ESI (*m/z*): $[M+H]^+$ Calcd for $C_{50}H_{78}NO_{12}$ 884.5524; Found 884.5519, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (d, *J* = 8.1 Hz, 2H), 6.89 (d, *J* = 10.8 Hz, 1H), 6.83 (d, *J* = 8.2 Hz, 2H), 6.46 (d, *J* = 10.9 Hz, 1H), 5.10 – 4.88 (m, 2H), 4.11 – 3.92 (m, 3H), 3.80 (d, *J* = 10.2 Hz, 1H)，3.79 (s, 3H), 3.62 (d, *J* = 9.9 Hz, 1H), 3.39 (t, *J* = 5.4 Hz, 1H), 2.88 (td, *J* = 11.1, 3.7 Hz, 1H), 2.73 – 2.61 (m, 2H), 2.57 (d, *J* = 9.8 Hz, 1H), 2.03 – 0.52 (m, 55H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 214.00, 177.64, 159.10, 130.84, 130.38, 129.46, 116.32, 113.58, 105.07, 98.49, 89.79, 75.68, 74.94, 74.89, 72.00, 71.55, 68.06, 55.35, 55.19, 49.37, 49.29, 40.35, 38.93, 36.38, 35.47, 32.85, 30.99, 30.16, 27.99, 26.33, 24.58, 22.67, 21.55, 19.80, 17.97, 16.13, 16.11, 13.96, 13.14, 12.94, 12.05, 10.89, 6.77, 6.67.

4-Trifluoromethyl Benzyl derivative 15

Yield 52%, white foamed solid, HRMS-ESI (*m/z*): $[M+H]^+$ Calcd for $C_{50}H_{75}NO_{11}F_3$ 922.5292, Found 922.5276, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (d, *J* = 8.0 Hz, 2H), 7.32 (d, *J* = 7.9 Hz, 2H), 6.91 (d, *J* = 10.8 Hz, 1H), 6.46 (d, *J* = 10.8 Hz, 1H), 4.03 (d, *J* = 10.2 Hz, 1H), 3.90 (dd, *J* = 11.0, 5.8 Hz, 1H), 3.78 (t, *J* = 7.2 Hz, 2H), 3.55 (d, *J* = 10.0 Hz, 1H), 3.31 (q, *J* = 3.9 Hz, 1H), 2.79 (td, *J* = 11.1, 3.6 Hz, 1H), 2.61 (ddd, *J* = 13.4, 9.2, 5.1 Hz, 2H), 2.52 (d, *J* = 10.6 Hz, 1H), 2.00 – 0.44 (m, 55H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 214.48 , 178.15 , 131.78 , 127.54 , 125.44 , 116.43 , 104.96 , 98.72 , 89.87 , 75.29 , 75.13 , 75.00 , 74.08 , 71.79 , 68.30 , 55.74 , 49.75 , 40.59 , 39.09 , 36.65 , 35.75 , 32.98 , 30.10 , 28.19 , 26.49 , 25.18 , 22.85 , 21.52 , 20.02 , 18.16 , 16.29 , 14.18 , 13.39 , 13.17 , 12.13 , 11.12 , 6.96 , 6.69 .

3-Trifluoromethyl Benzyl derivative 16

Yield 55%, white foamed solid, HRMS-ESI (*m/z*): $[M+Na]^+$ Calcd for $C_{50}H_{74}F_3NO_{11}Na$ 944.5112; Found 944.5102, ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.53 (dd, *J* = 28.7, 9.5 Hz, 4H), 6.98 (d, *J* = 10.8 Hz, 1H), 6.56 (d, *J* = 11.0 Hz, 1H), 5.25 – 5.07 (m, 2H), 4.18 – 4.08 (m, 1H), 3.99 (ddd, *J* = 19.8, 12.5, 6.4 Hz, 2H), 3.86 (d, *J* = 10.2 Hz, 1H), 3.66 (d, *J* = 9.9 Hz, 1H), 3.42 (q, *J* = 4.5 Hz, 2H), 2.91 (td, *J* = 11.0, 3.7 Hz, 1H), 2.83 – 2.55 (m, 3H), 2.20 – 0.42 (m, 61H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 214.48, 178.15, 131.78, 127.54, 125.44, 116.43, 104.96, 98.72, 89.87, 75.89, 75.29, 75.13, 75.00, 74.08, 71.79, 68.30, 55.74, 49.75, 40.59, 39.09, 36.65, 35.75, 32.98, 30.10, 28.19, 26.49, 25.18, 22.85, 21.52, 20.02, 18.16, 16.29, 14.18, 13.39, 13.17, 12.13, 11.12, 6.96, 6.69.

4-Methyl Benzyl derivative 17 (Sodium salt)

Yield 48%, white foamed solid, HRMS-ESI (*m/z*): $[M+Na]^+$ Calcd for $C_{50}H_{77}NO_{11}Na$ 890.5389; Found 890.5389, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 (d, *J* = 7.7 Hz, 2H), 7.10 (d, *J* = 7.7 Hz, 2H), 6.91 (d, *J* = 10.8 Hz,

1H), 6.46 (d, *J* = 10.8 Hz, 1H), 5.15 – 4.92 (m, 2H), 4.06 (d, *J* = 10.4 Hz, 1H), 4.03 – 3.92 (m, 2H), 3.80 (d, *J* = 10.2 Hz, 1H), 3.63 (d, *J* = 9.9 Hz, 1H), 3.38 (t, *J* = 5.5 Hz, 1H), 2.89 (td, *J* = 11.1, 3.8 Hz, 1H), 2.74 – 2.61 (m, 2H), 2.57 (d, *J* = 10.5 Hz, 1H), 2.32 (s, 3H), 2.04 – 0.49 (m, 55H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 216.66, 183.78, 146.48, 137.01, 134.89, 130.17, 128.94, 128.55, 128.40, 117.06, 105.10, 98.75, 88.69, 76.28, 76.09, 75.22, 74.27, 71.33, 69.64, 67.04, 55.20, 51.17, 50.42, 40.23, 38.51, 35.89, 35.02, 33.10, 32.41, 32.33, 29.34, 28.07, 27.50, 27.03, 23.68, 21.22, 20.30, 20.14, 17.46, 15.99, 15.95, 14.62, 13.12, 12.59, 11.99, 10.80, 6.88, 6.56.

4-Trifluoromethoxyl Benzyl derivative 18

Yield 63%, white foamed solid, HRMS-ESI (*m/z*): $[M+H]^+$ Calcd for $C_{50}H_{75}NO_{12}F_3$ 938.5241; Found 938.5254, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (d, *J* = 8.2 Hz, 1H), 7.13 (dd, *J* = 19.5, 8.1 Hz, 2H), 6.93 (d, *J* = 10.9 Hz, 1H), 6.51 (d, *J* = 10.9 Hz, 1H), 5.16 – 4.96 (m, 2H), 4.07 (d, *J* = 10.2 Hz, 1H), 3.97 (dd, *J* = 10.1, 5.9 Hz, 1H), 3.81 (d, *J* = 10.0 Hz, 1H), 3.62 (d, *J* = 10.1 Hz, 1H), 3.40 (d, *J* = 6.2 Hz, 1H), 2.87 (td, *J* = 11.0, 3.7 Hz, 1H), 2.77 – 2.50 (m, 2H), 2.08 – 0.46 (m, 51H). ^{13}C NMR (101 MHz, $CDCl_3$) δ213.86 , 177.73 , 131.51 , 128.93 , 120.74 , 116.09 , 105.03 , 98.49 , 90.03 , 74.92 , 74.87 , 72.07 , 71.57 , 68.06 , 55.29 , 49.40 , 49.33 , 40.32 , 38.97 , 36.40 , 35.58 , 34.26 , 32.84 , 30.19 , 28.00 , 26.34 , 24.64 , 22.63 , 21.56 , 19.80 , 17.97 , 16.09 , 16.04 , 13.94 , 13.13 , 12.96 , 11.92 , 10.88 , 6.77 , 6.58 .

4-Nitro Benzyl derivative 19 (Sodium salt)

Yield 58%, white foamed solid, HRMS-ESI (*m/z*): $[M+Na]^+$ Calcd for $C_{49}H_{74}N_2O_{13}Na$ 921.5089; Found 921.5112,^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, *J* = 8.3 Hz, 2H), 7.44 (d, *J* = 8.4 Hz, 2H), 6.97 (d, *J* = 10.8 Hz, 1H), 6.55 (d, *J* = 10.9 Hz, 1H), 5.16 (q, *J* = 13.0 Hz, 2H), 4.17 – 4.04 (m, 1H), 3.95 (dq, *J* = 25.1, 6.8, 6.3 Hz, 2H), 3.82 (d, *J* = 10.1 Hz, 1H), 3.62 (d, *J* = 9.9 Hz, 1H), 3.37 (dd, *J* = 7.0, 4.2 Hz, 1H), 2.86 (td, *J* = 11.0, 3.6 Hz, 1H), 2.75 – 2.53 (m, 3H), 2.06 – 0.60 (m, 55H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 216.54, 184.17, 147.64, 147.14, 146.01, 131.60, 127.93, 123.88, 116.63, 104.82, 98.77, 89.00, 76.16, 75.88, 75.43, 74.50, 74.20, 71.40, 69.81, 67.10, 55.14, 53.46, 51.22, 50.28, 40.20, 38.53, 35.92, 34.69, 33.00, 32.41, 32.29, 29.12, 28.01, 27.65, 26.96, 23.77, 20.26, 20.02, 17.42, 15.95, 15.89, 14.57, 13.09, 12.54, 11.99, 10.70, 6.83, 6.53.

2. Biological

In vitro cytotoxicity evaluation

Each test solution for *in vitro* assay was prepared by diluting with DMSO (Sigma-Aldrich). Solutions for *in vitro* assay were dissolved by culture medium RPMI 1640 (Gibco) supplemented with 10% FBS (Gibco), 100 U/mL penicillin and 100 µg/mL streptomycin (Gibco) to obtain a series of concentrations. All cells (HT-29 colorectal cancer, HGC-27 gastric cancer, triple negative MDA-MB-231 human breast cancer cells) used in the research were prepared at 3.5×10^4 cells/mL concentration and each 100 µL cells suspension was seeded in 96-well cell microplates (Corning) for 24 hours (37°C, 5%CO_2). Then each solution was added and incubated for another 48 hours. For the control group, equivalent concentration of DMSO (final concentration 0.1%) was added. **MTT** (3-[4,5-dimethylthiazol-2yl]-diphenyl tetrazoliumbromide) (Sigma-Aldrich) method was employed to measure the number of surviving cells and recorded the OD values at 570 nm using microplate reader (Perkin Elmer). The IC_{50} values were calculated using Prism Graphpad software.

In vitro cell viability assay

SH-SY5Y cells were maintained in DMEM supplemented with 10%-15% foetal bovine serum (FBS) and 100 units penicillin and 100 µg/mL streptomycin and cultured at 37 °C with 5% CO_2. Each test solution of compound was prepared by diluting with DMSO. SH-SY5Y were seeded into 96-well plates at 2 x 10^4 cell/well and then treated with test solution. At the end of the incubation period, MTT solution (final concentration: 0.5 mg/mL) was added to each well following which the cells were incubated for 4 hours at 37 °C. After removing the medium, DMSO was added to dissolve the blue formazan product. Absorbance was measured with a microplate reader at 490 nm. Cell survival rate were expressed as the percentage of the absorbance of treated cells to that of control cells.

Intracellular calcium detection through flow cytometry

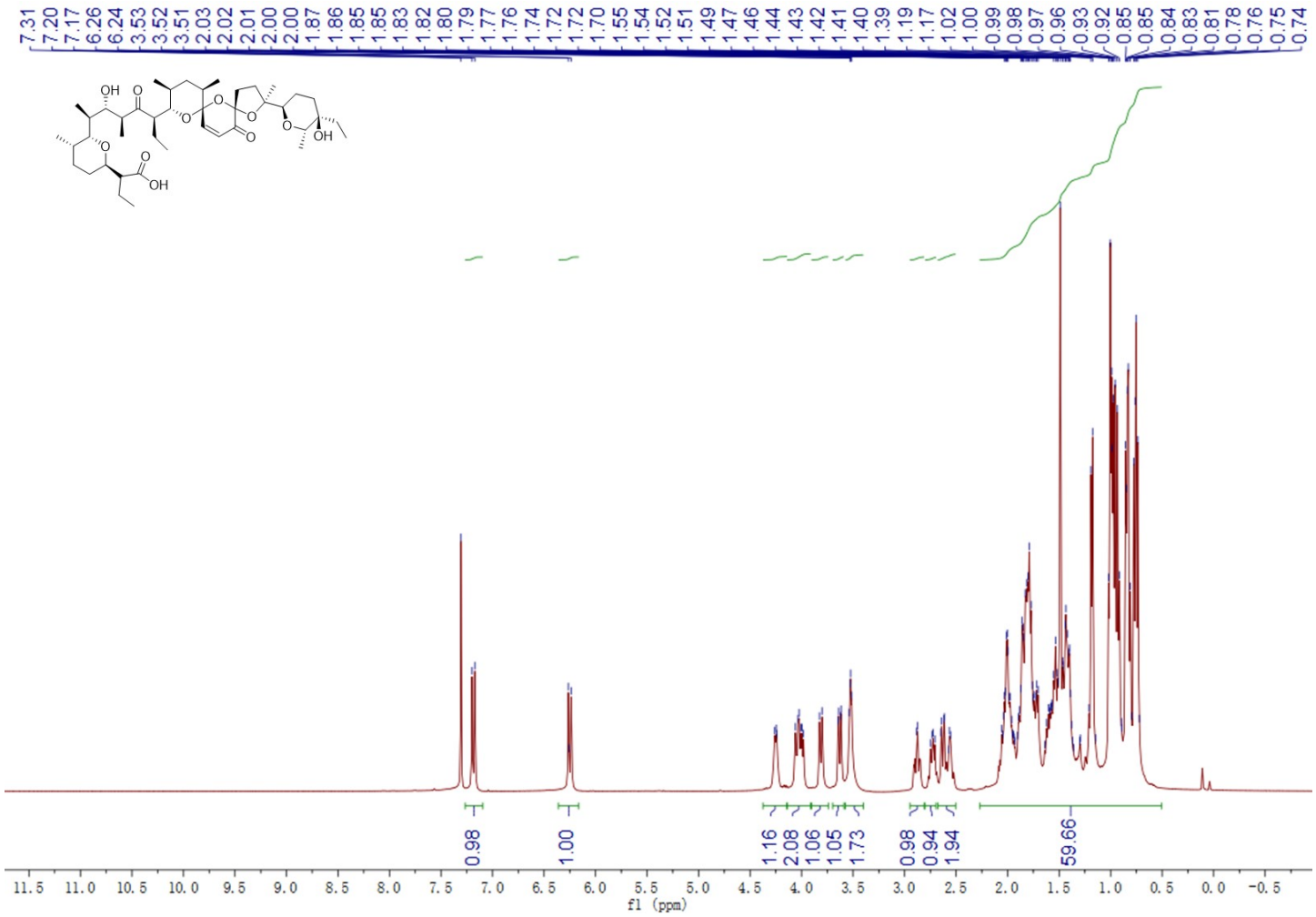
HT-29 cells were seeded in a six-well plate at a density of 1×10^5 cells overnight and then salinomycin (5 µM) and compound **6** (0.5 µM) were added into the culture medium to incubate 24 hours, the cells were then rinsed 3 times with 1 mL of calcium-free HEPES buffer (as described above) and loaded with 1mL HEPES buffer containing 1µM Fluo-3, AM and 0.1% pluronic F-127 at 37°C for 30 min. After loading, the cells were detached from the plate by using 0.125% EDTA-Trypsin, the cells were then resuspended and centrifuged for 5 min at 2000 rpm, remove the

supernatant and resuspended with 500 μL calcium-free HEPES buffer. The Samples were analyzed by BD Accuri C6 flow cytometry.

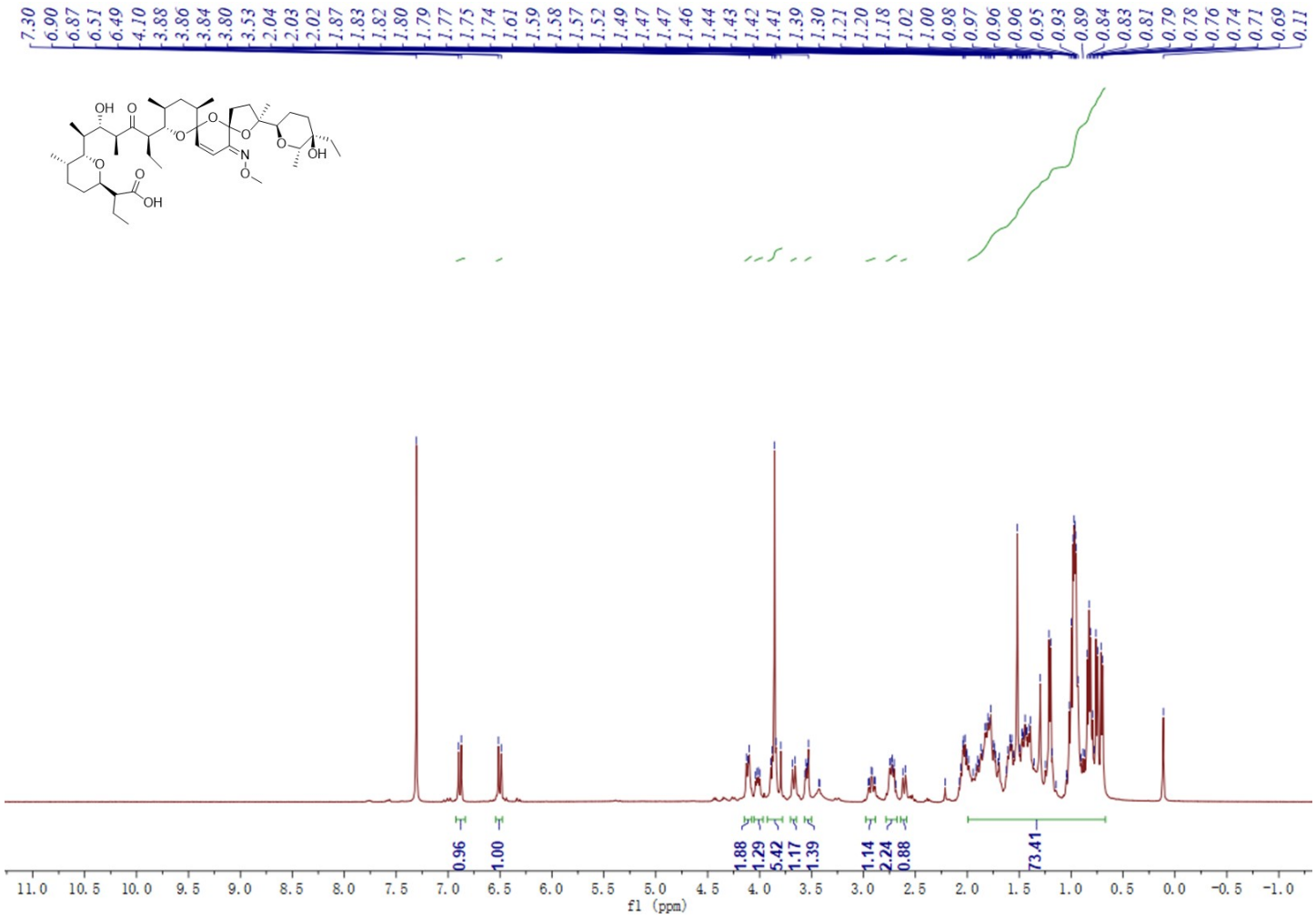
Life cell imaging of ER calcium release

HT-29 cells were seeded on cover slips in a six-well plate at a density of 1×10^5 cells overnight, and then the cells were incubated with salinomycin (5 μM) and compound **6** (0.5 μM) for another 24 hours. After treatment, the culture medium was removed and then washed 3 times with 1 mL of calcium-free HEPES buffer (130 mM NaCl, 5.0 mM KCl, 0.9 mM KH_2PO_4, 25 mM HEPES, 1 mM $MgCl_2$ 1 mM glucose and 0.25 μM sulfinpyrazone, pH 7.4). Afterwards, cells were loaded with 1mL HEPES buffer containing 4 μM Fluo-4 AM and 0.02% pluronic F-127 at 37 ^oC for 20 min, after loading, the cells were rinsed in calcium-free HEPES buffer to allow de-esterification of intracellular Fluo-4 AM for 5 min at room temperature. The cover slips were transferred to the laser-scanning confocal microscope (Nikon A1R SI) to record the fluorescence intensity at wavelength of 488 nm for 8 min. The baseline fluorescence intensity of cells was record in calcium-free HEPES buffer for 3 min, at 180s time point, 100μM ATP (a calcium releasing agonist) and 4 μM thapsigargin (a sarco/endoplasmic reticulum Ca^{2+}-ATPases inhibitor) was added to deplete the calcium of ER. Results are shown as (ΔF/F0), where F0 is the mean of the intensities from 0 s to180 s, and the ΔF is maximum fluorescence subtract F0.

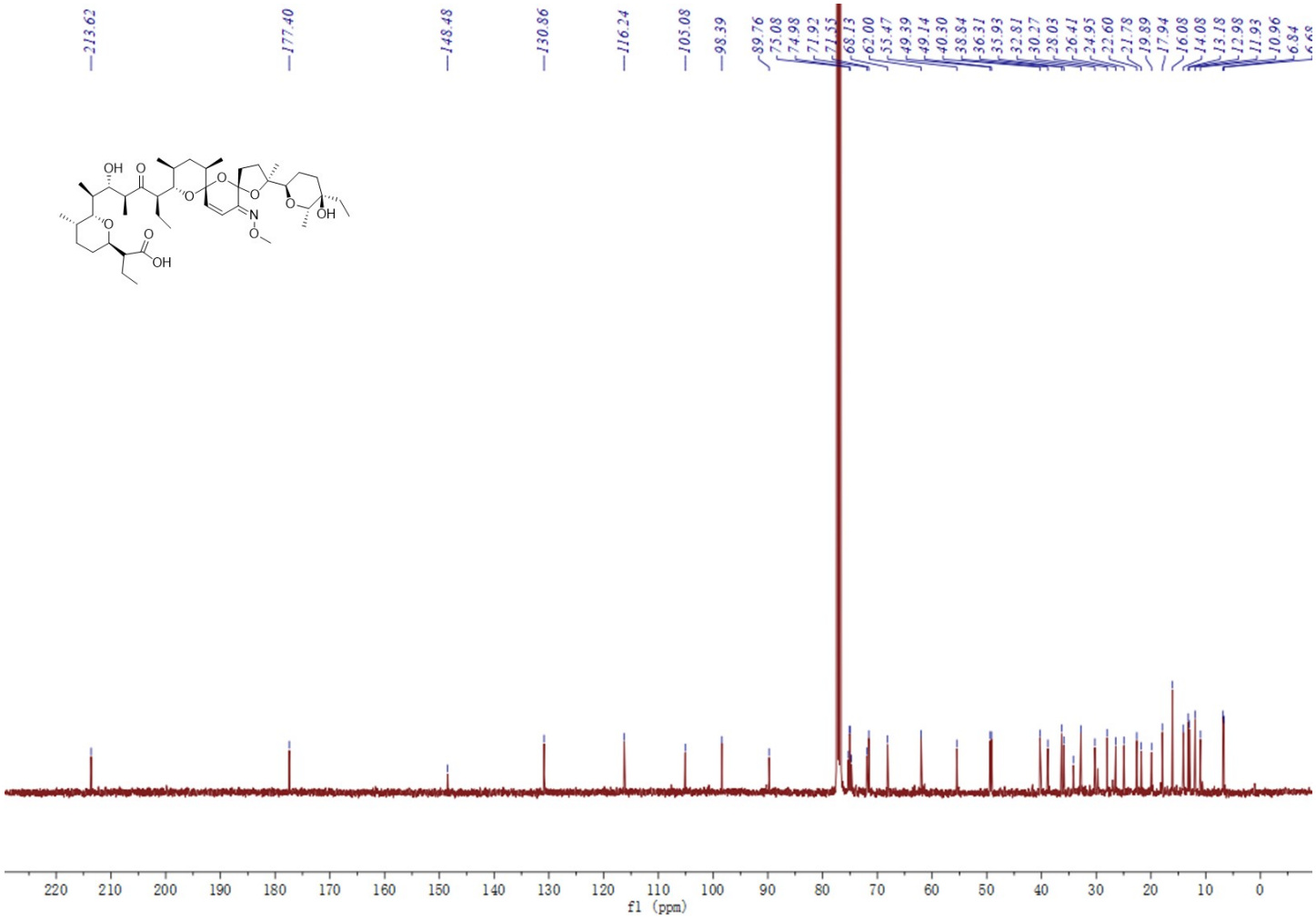
^{1}H NMR spectrum of compound **2** in $CDCl_3$



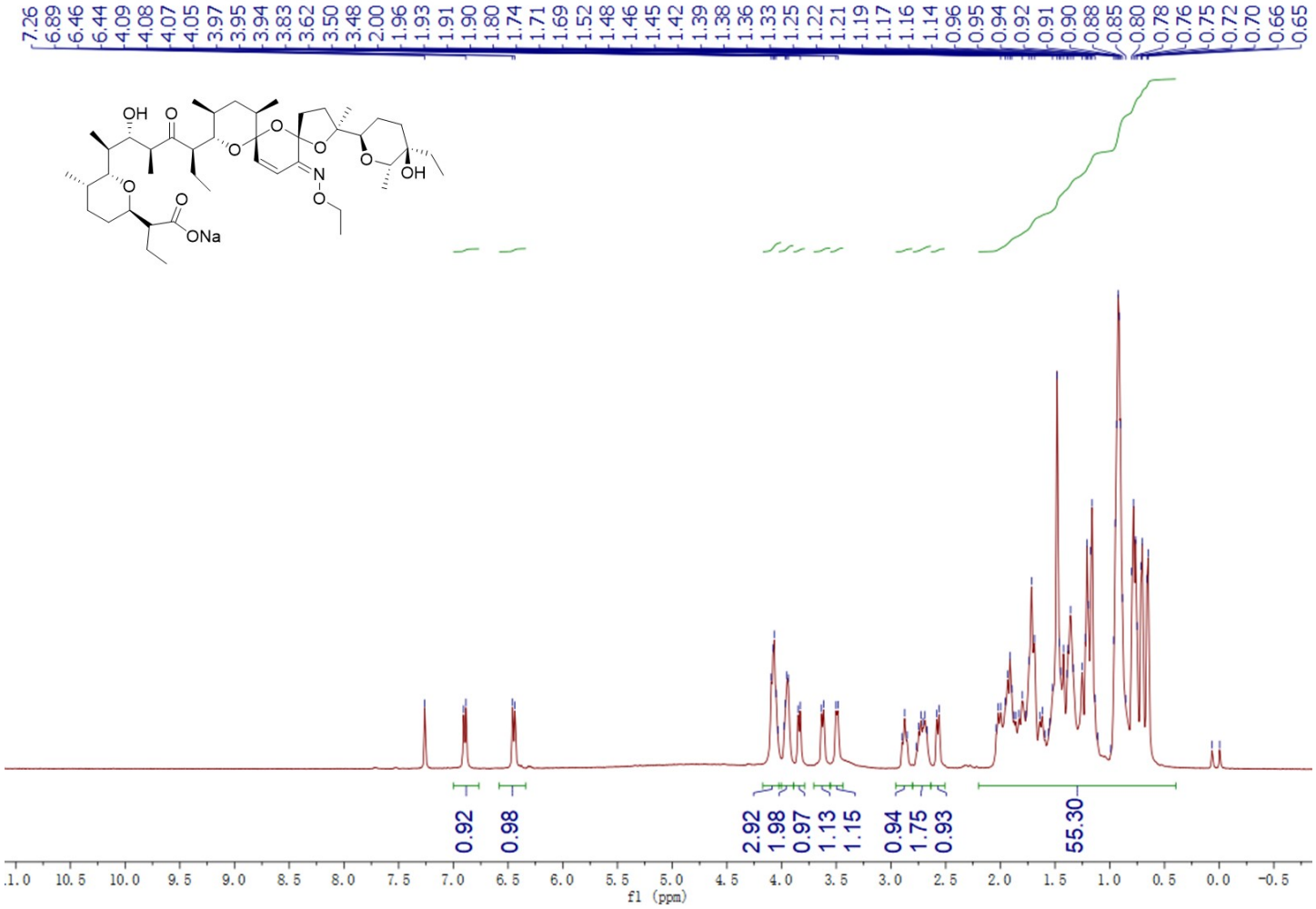
^{1}H NMR spectrum of compound **3** in $CDCl_3$



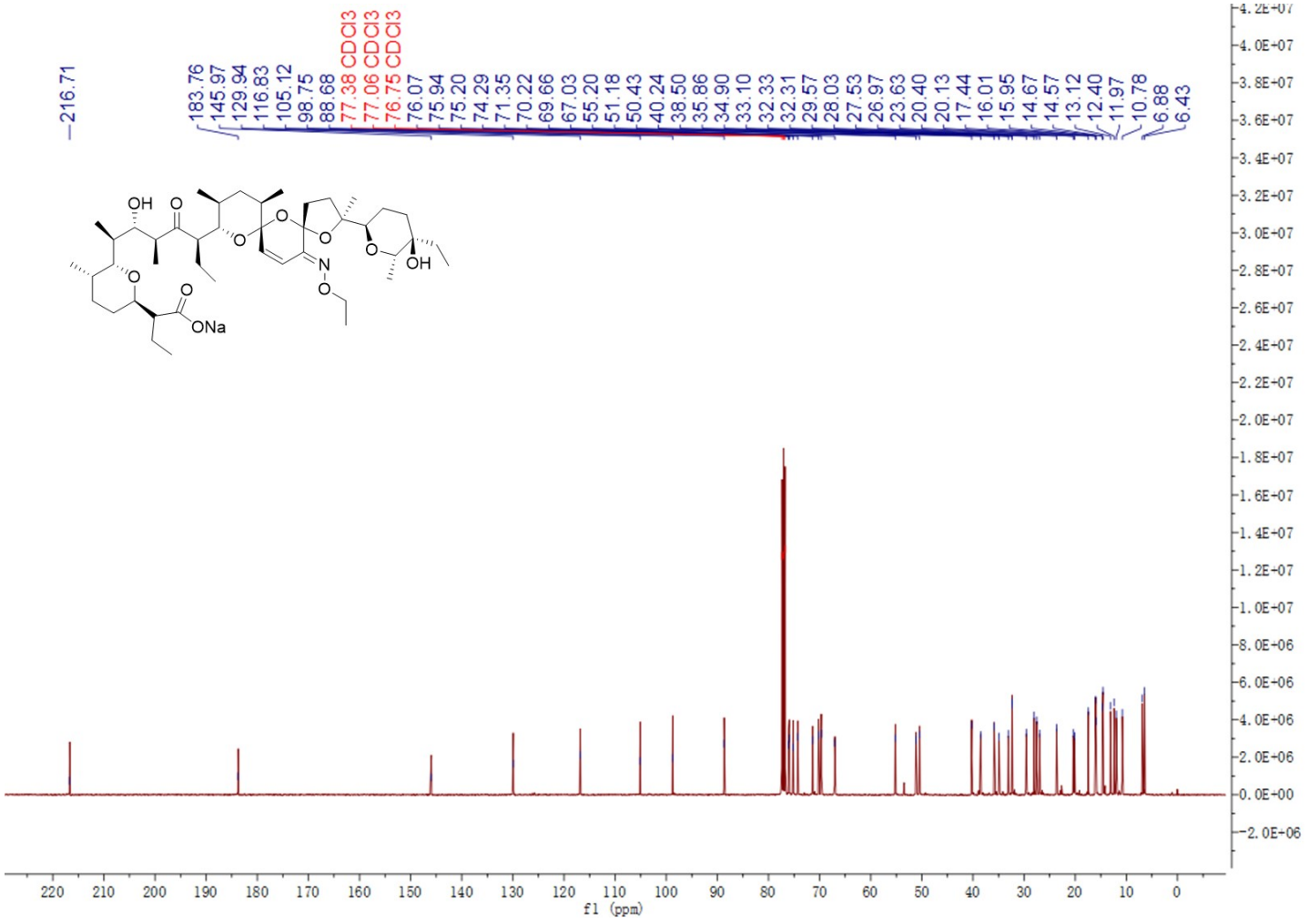
^{13}C NMR spectrum of compound **3** in $CDCl_3$



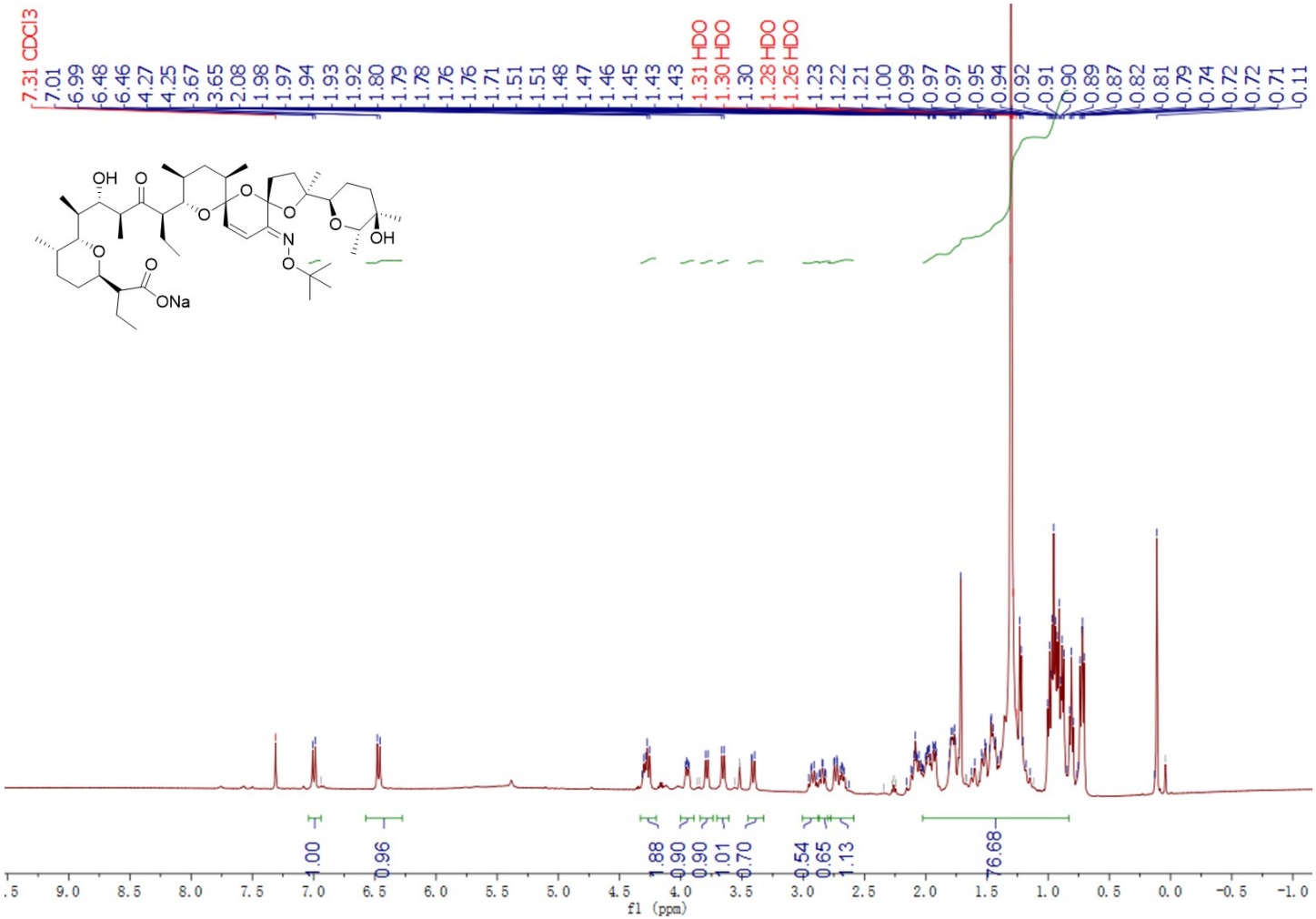
^{1}H NMR spectrum of compound **4** in $CDCl_3$



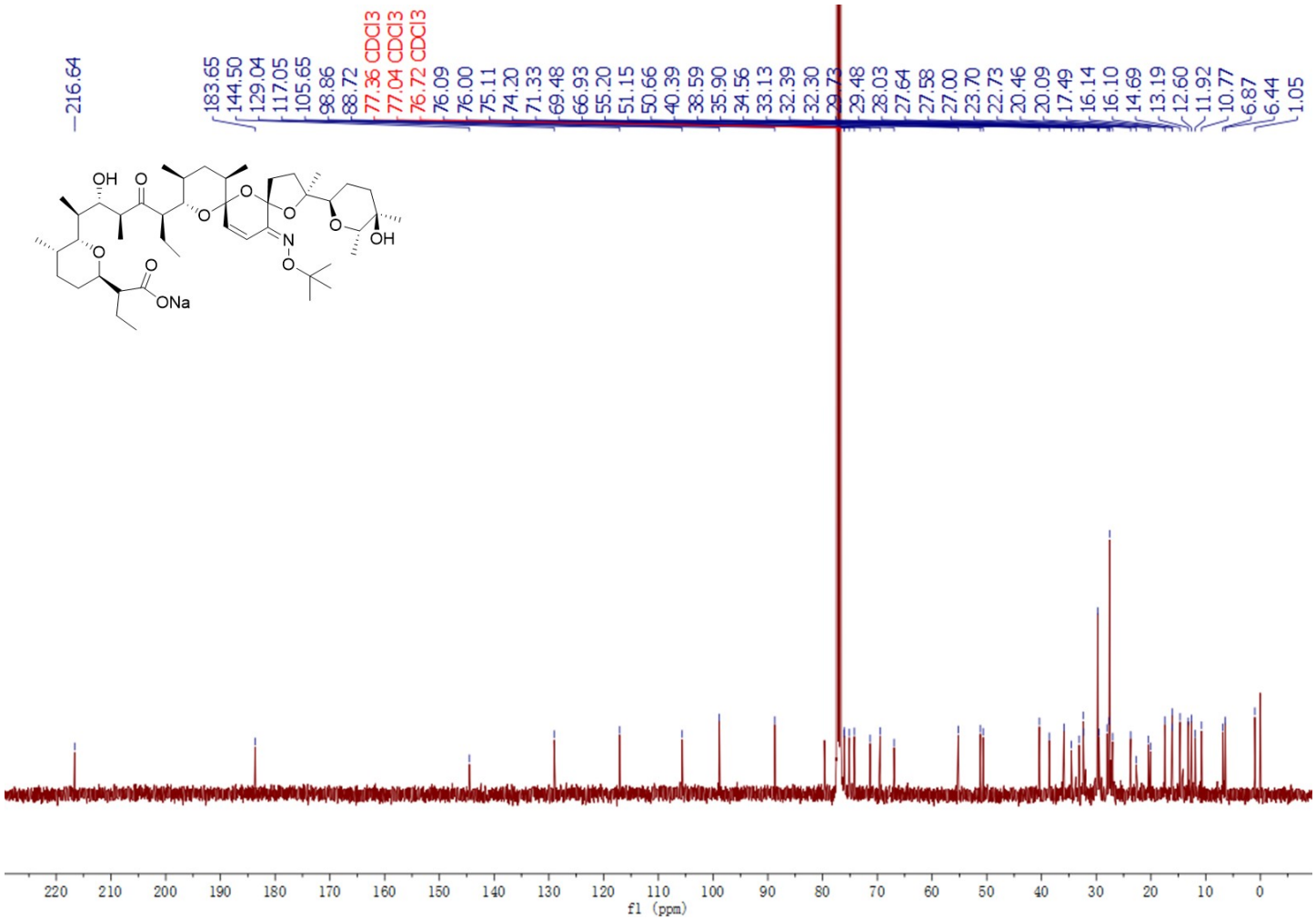
^{13}C NMR spectrum of compound **4** in $CDCl_3$



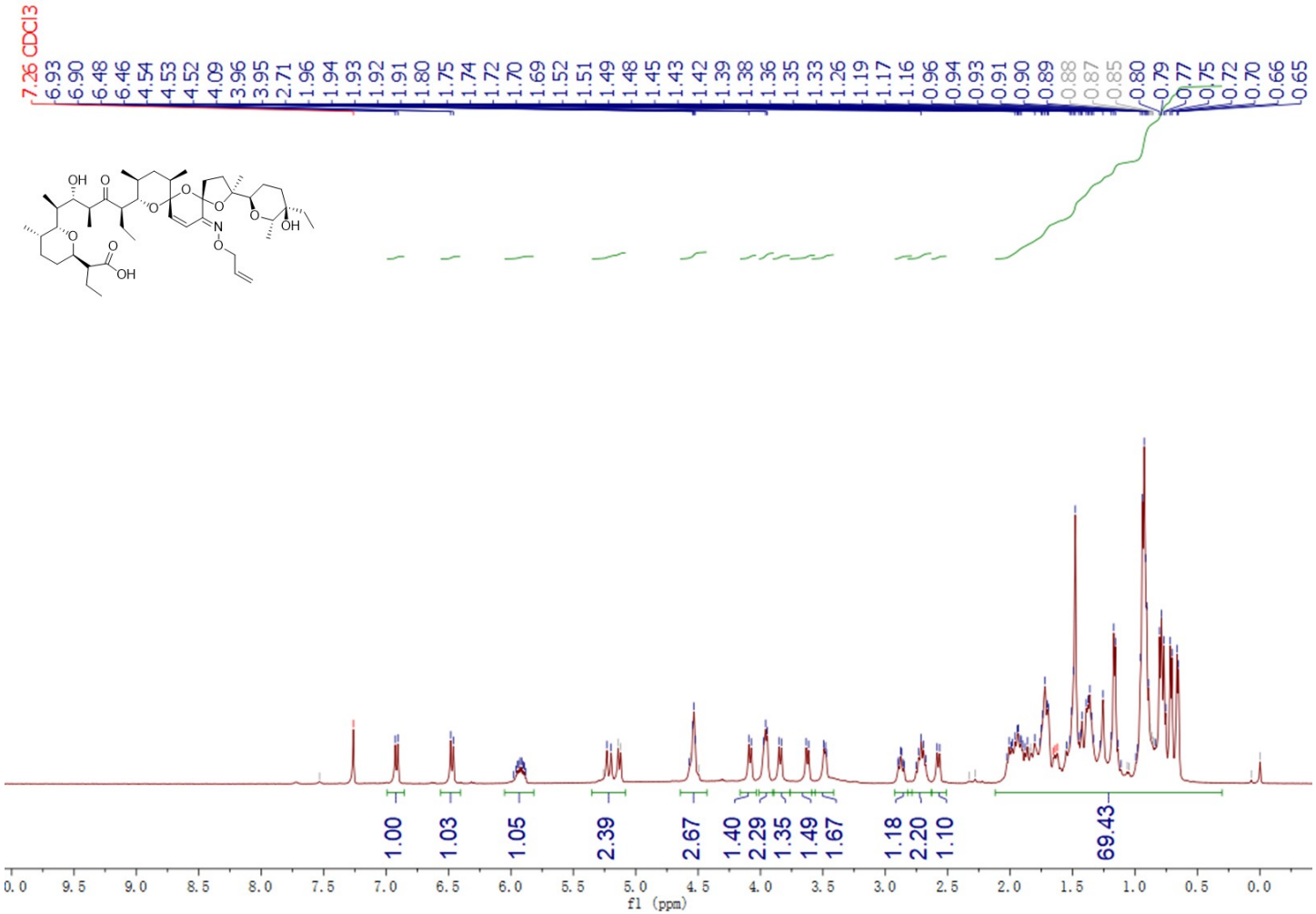
^{1}H NMR spectrum of compound **5** in $CDCl_3$



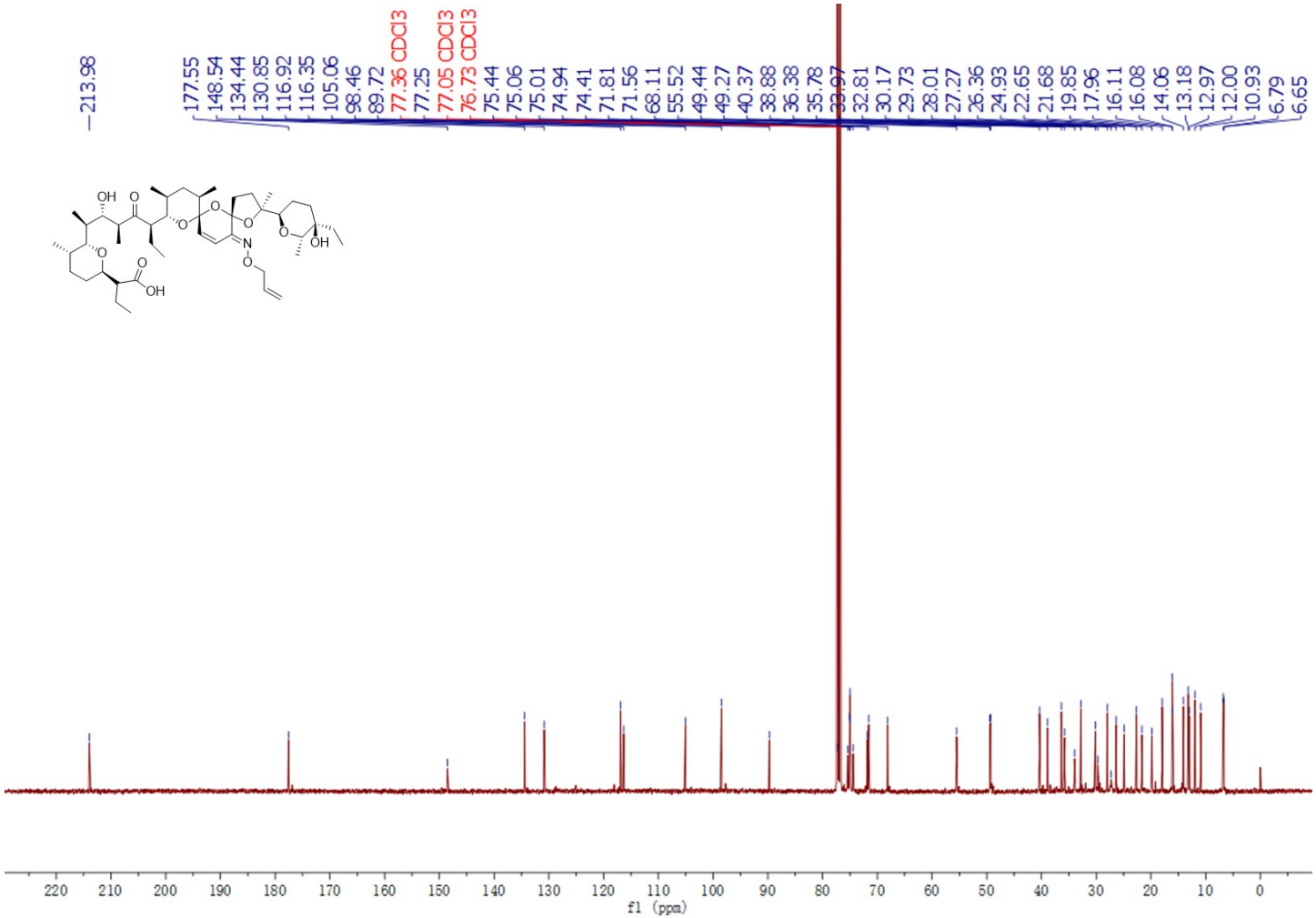
^{13}C NMR spectrum of compound **5** in $CDCl_3$



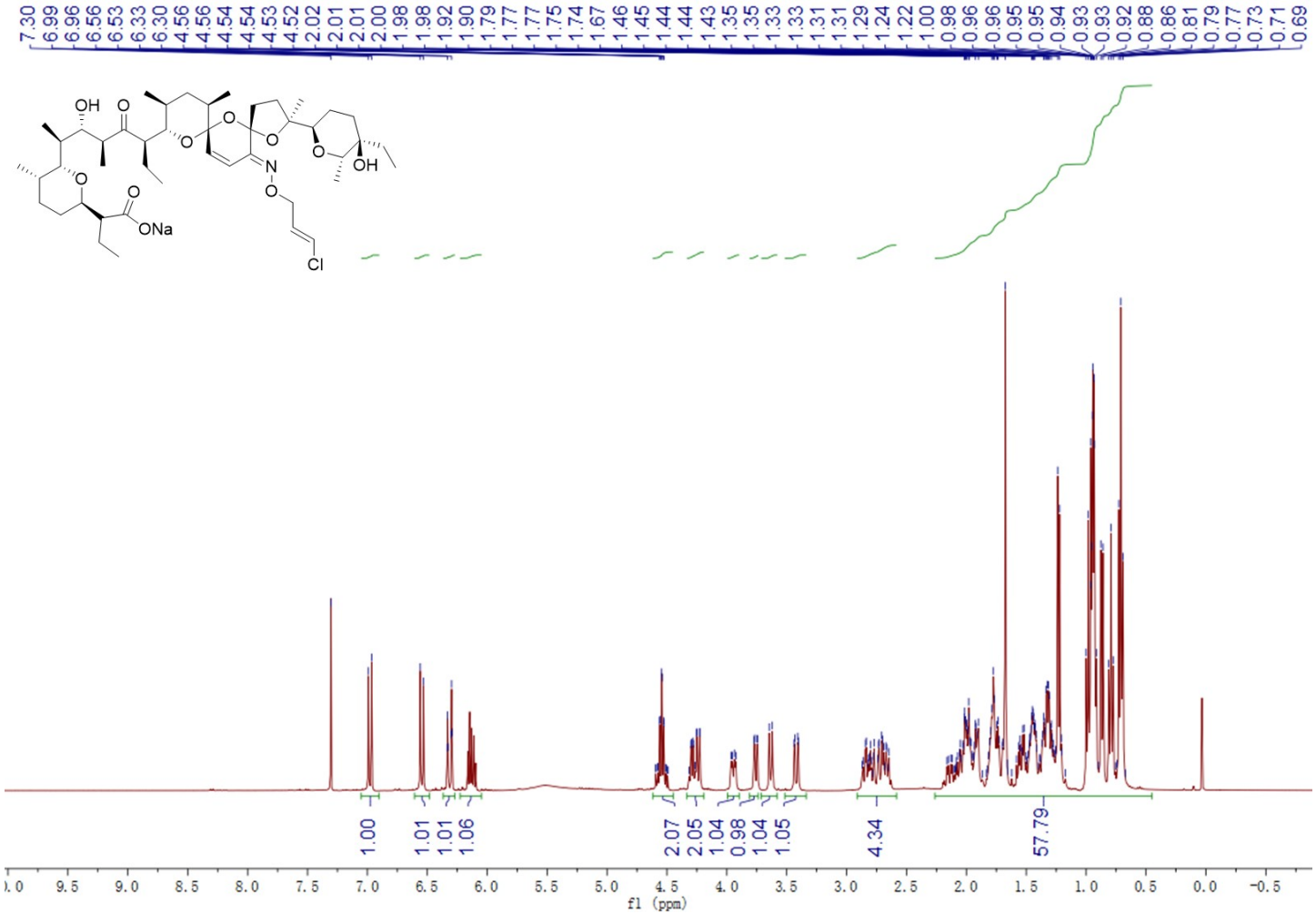
^{1}H NMR spectrum of compound **6** in $CDCl_3$



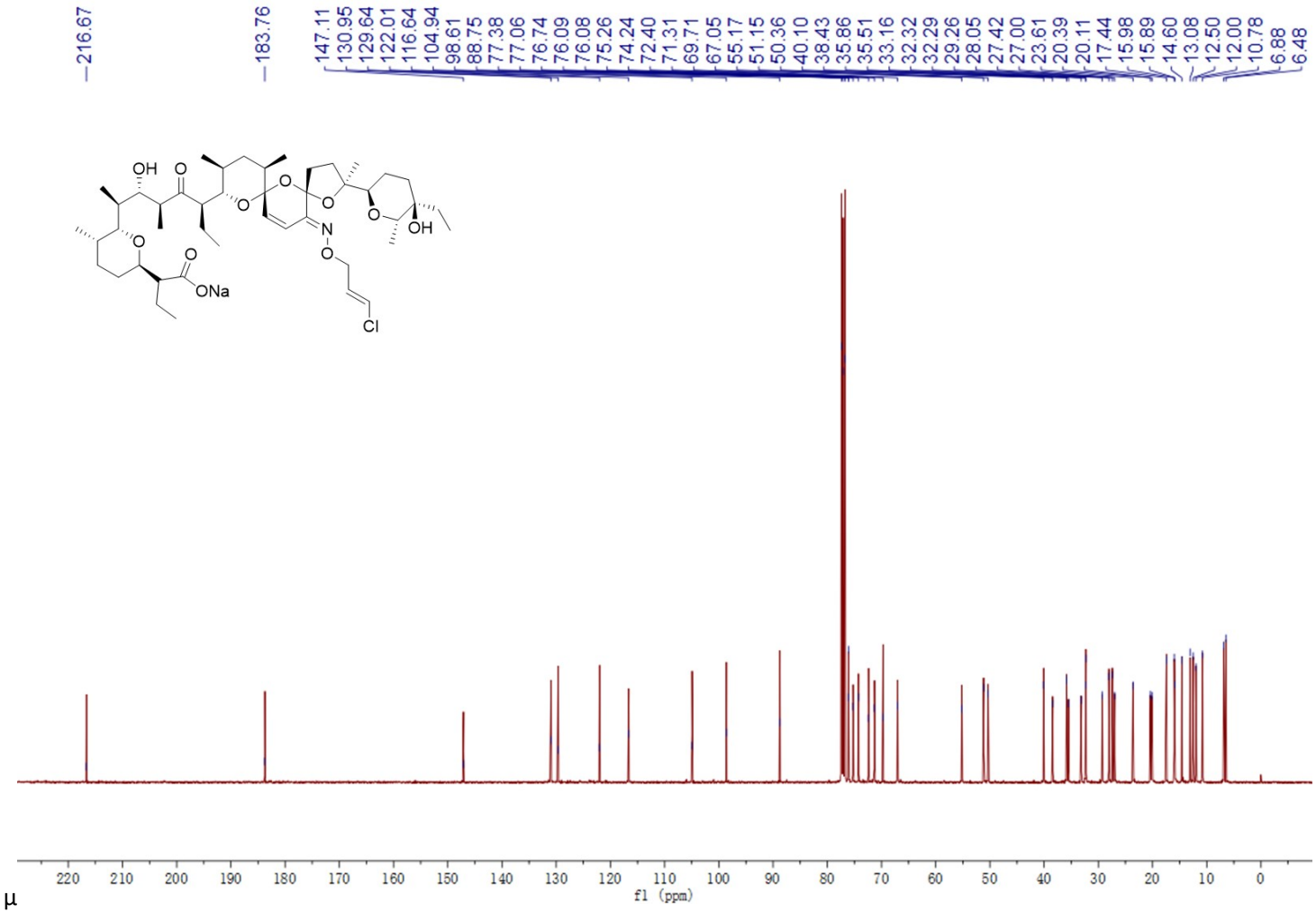
^{13}C NMR spectrum of compound **6** in $CDCl_3$



^{1}H NMR spectrum of compound **7** in $CDCl_3$

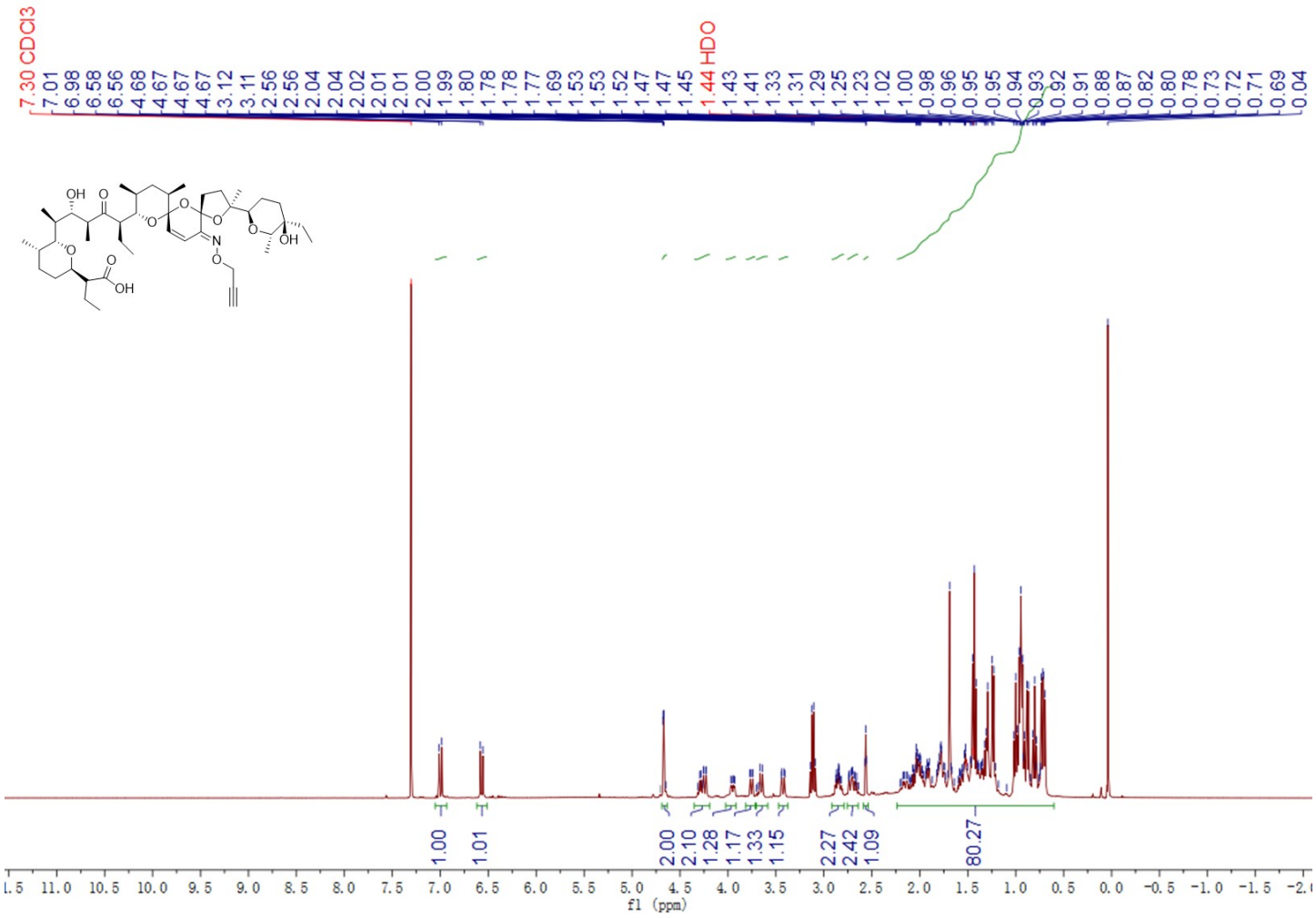


^{13}C NMR spectrum of compound **7** in $CDCl_3$

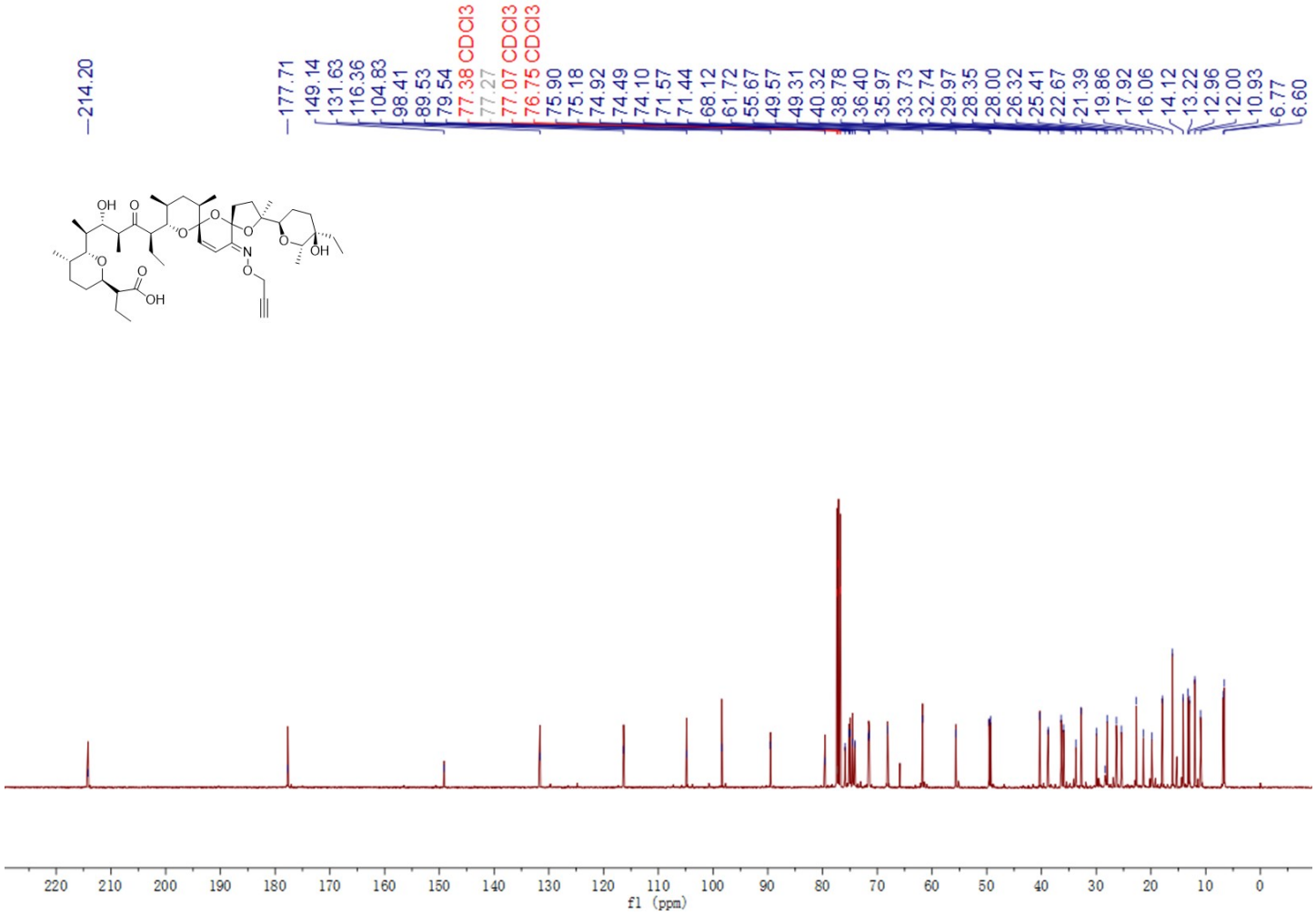


μ

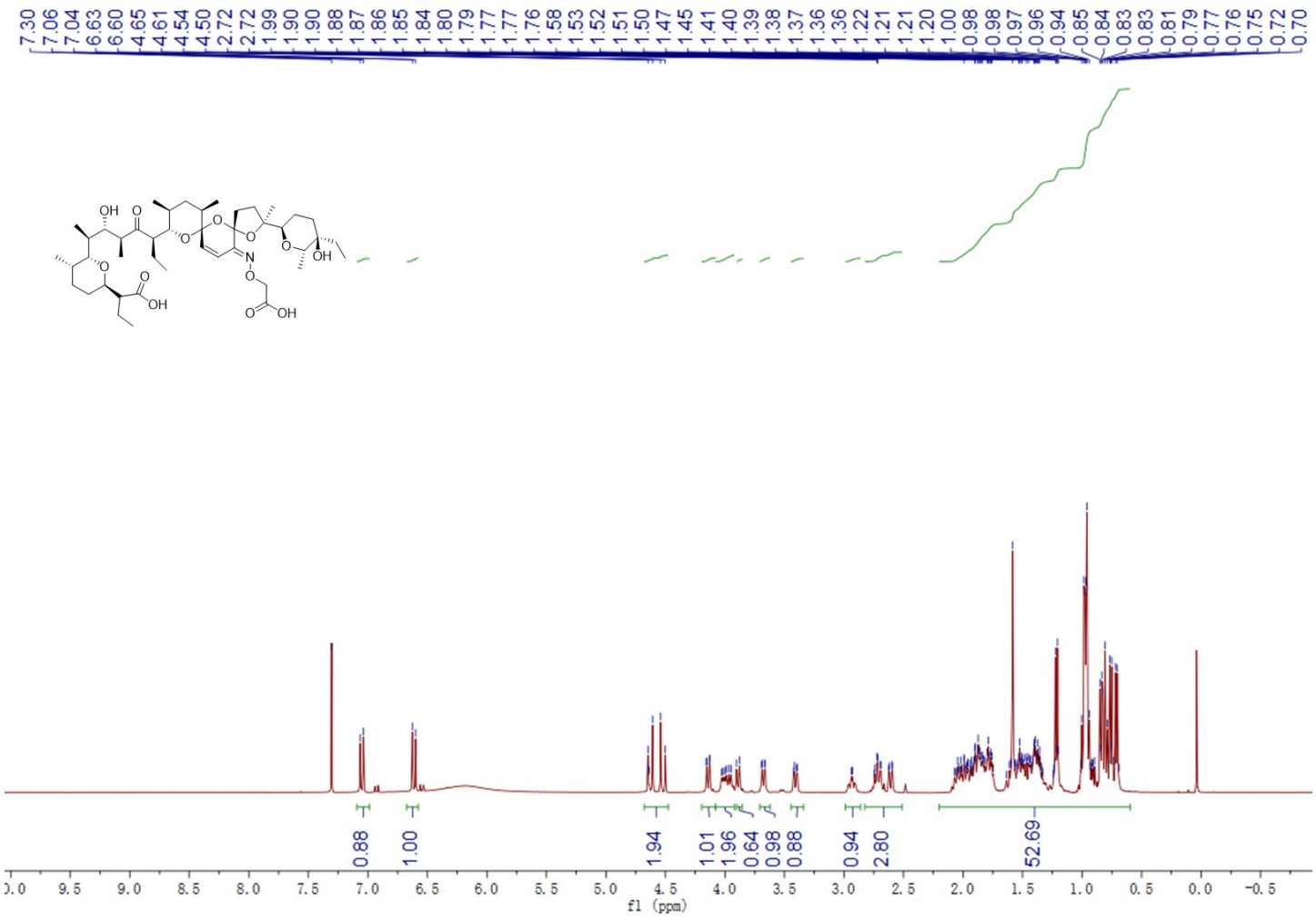
^{1}H NMR spectrum of compound **8** in $CDCl_3$



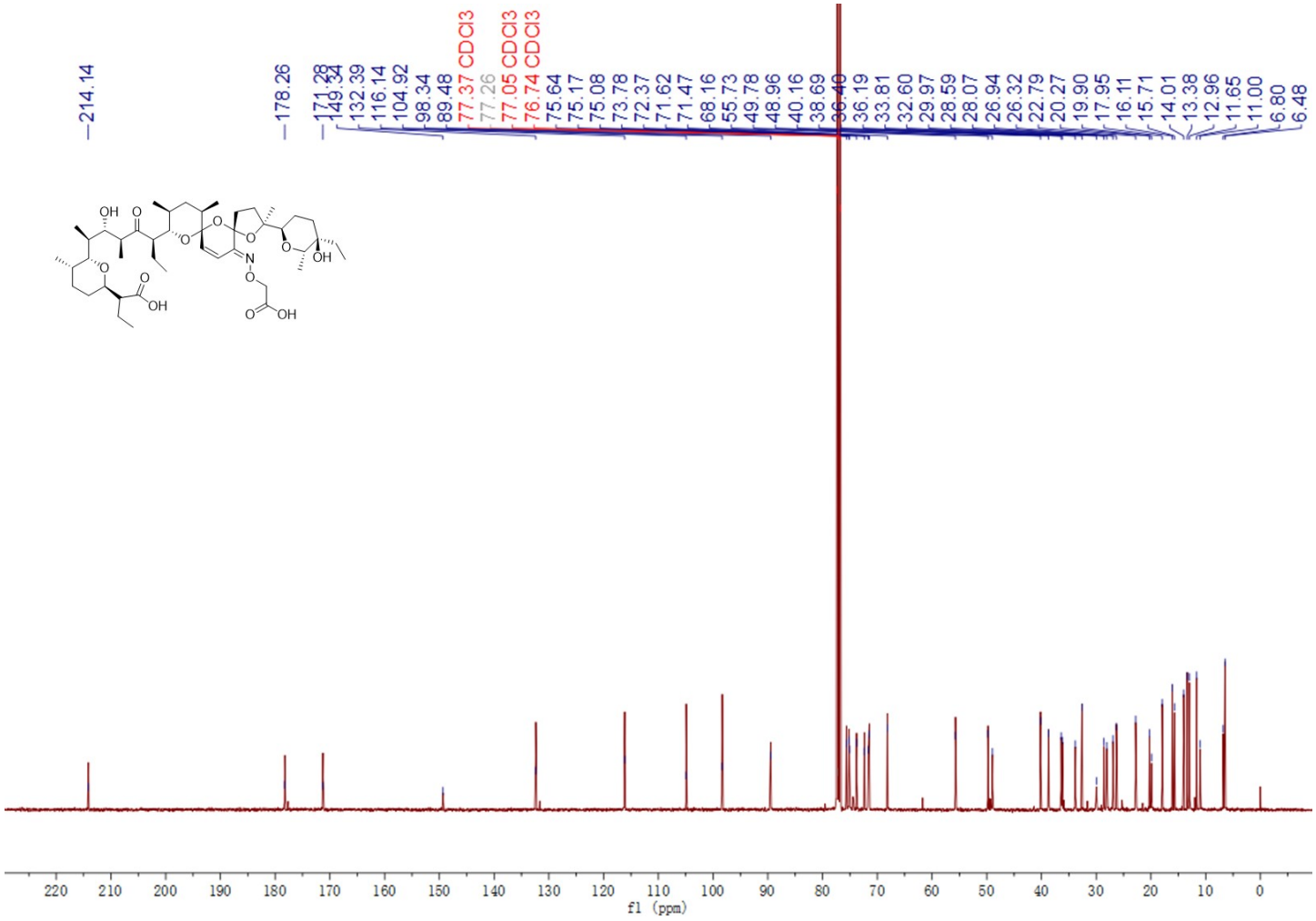
^{13}C NMR spectrum of compound **8** in $CDCl_3$



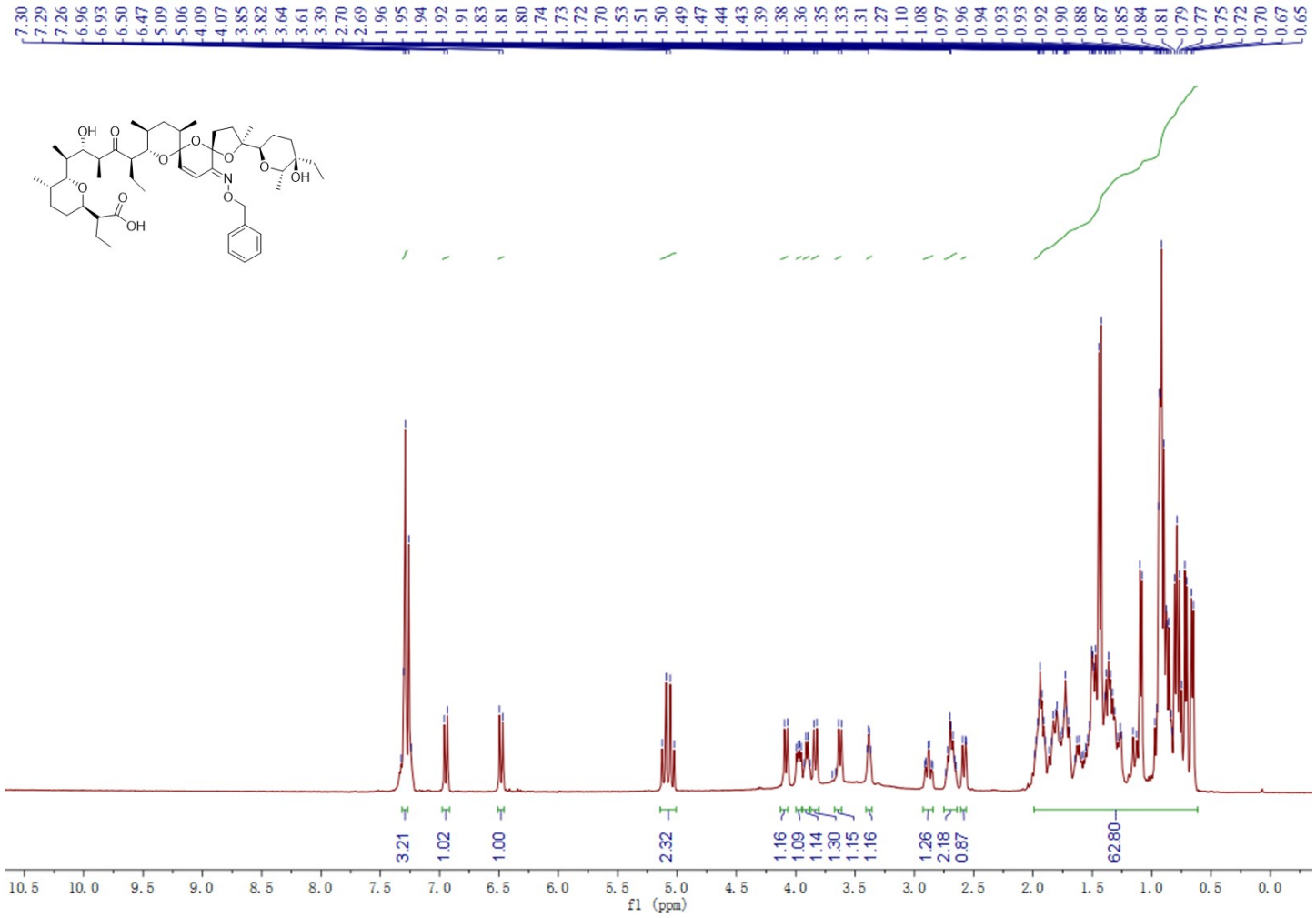
^{1}H NMR spectrum of compound **9** in $CDCl_3$



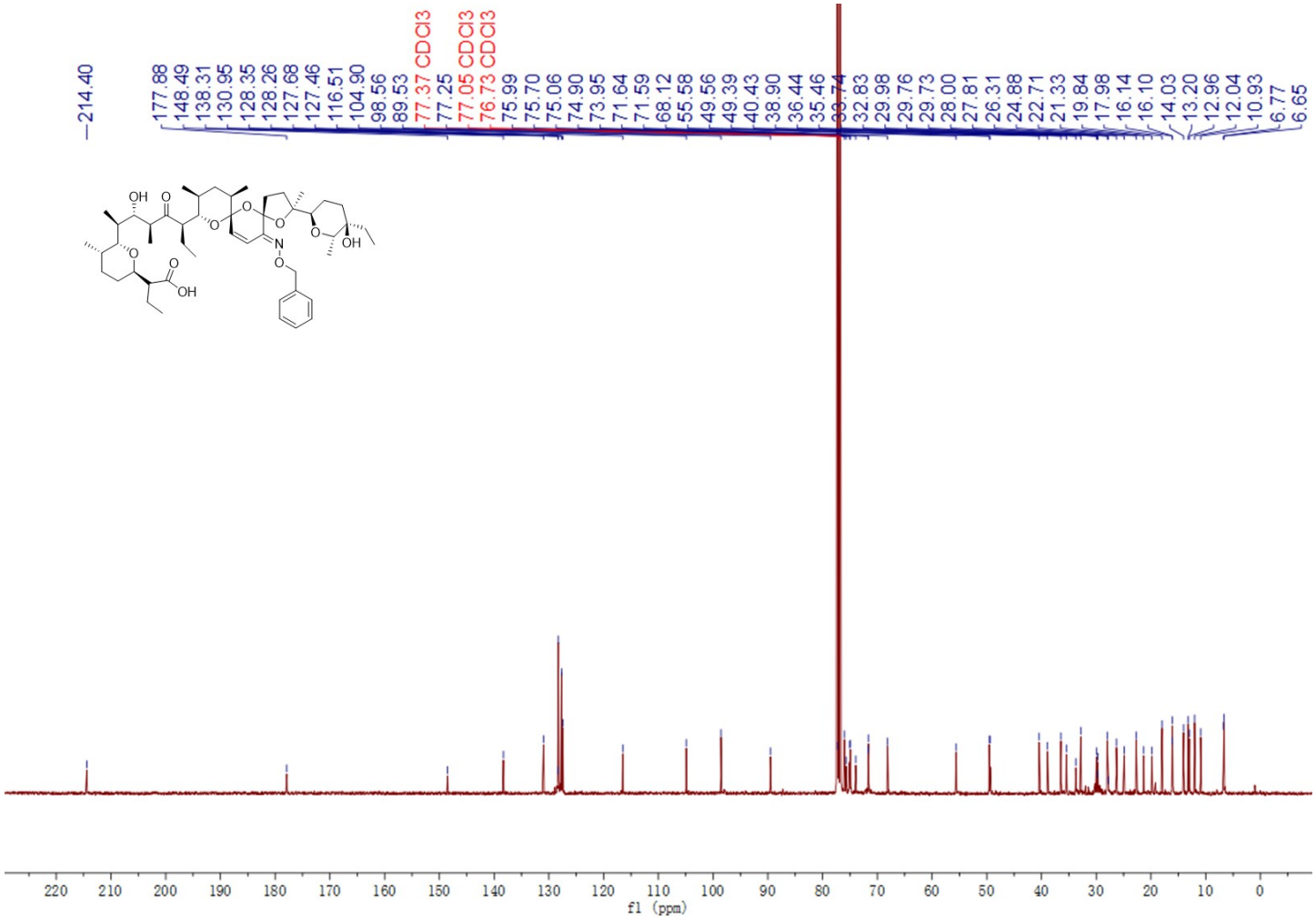
^{13}C NMR spectrum of compound **9** in $CDCl_3$



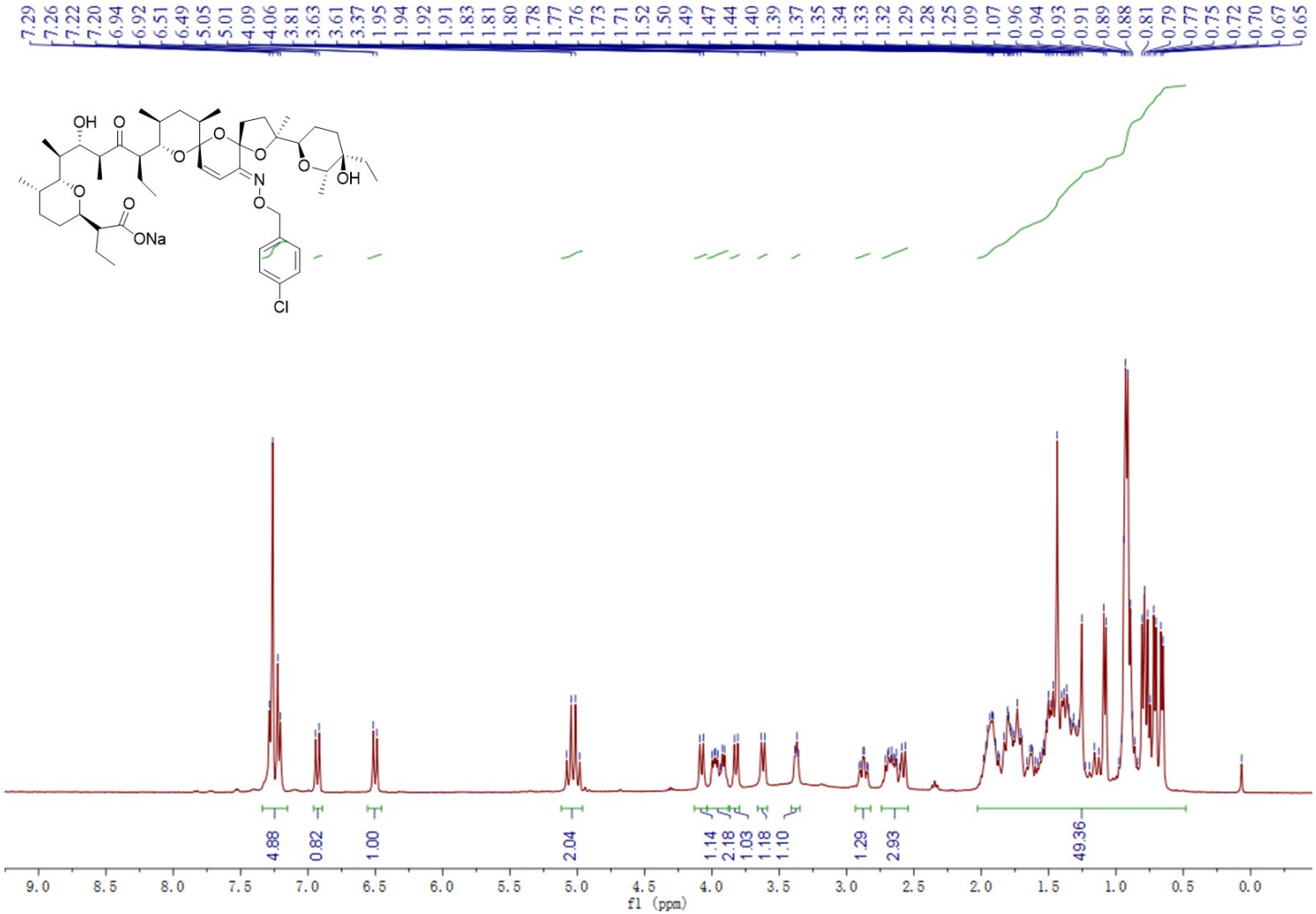
^{1}H NMR spectrum of compound **10** in $CDCl_3$



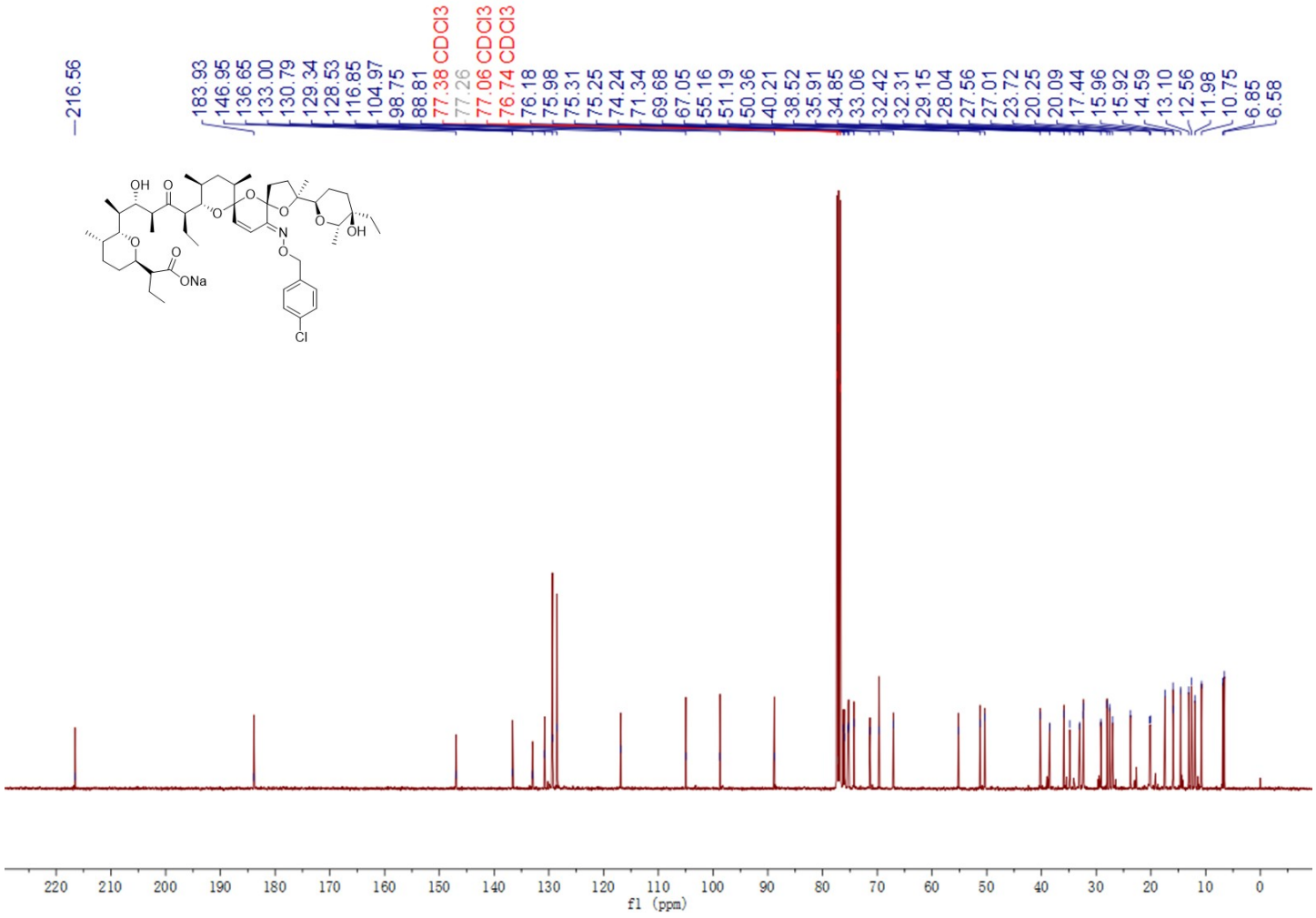
^{13}C NMR spectrum of compound **10** in $CDCl_3$



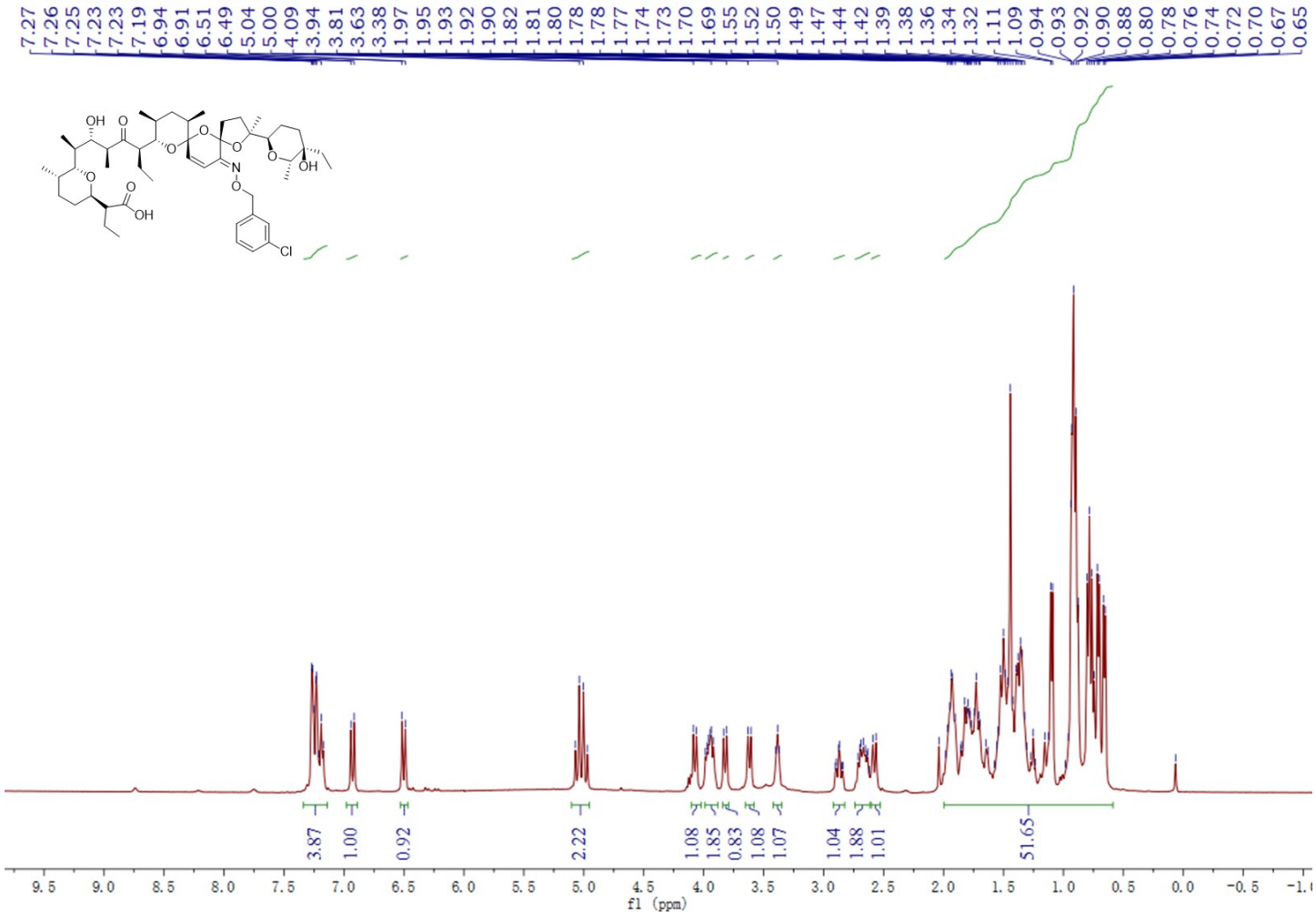
^{1}H NMR spectrum of compound **11** in $CDCl_3$



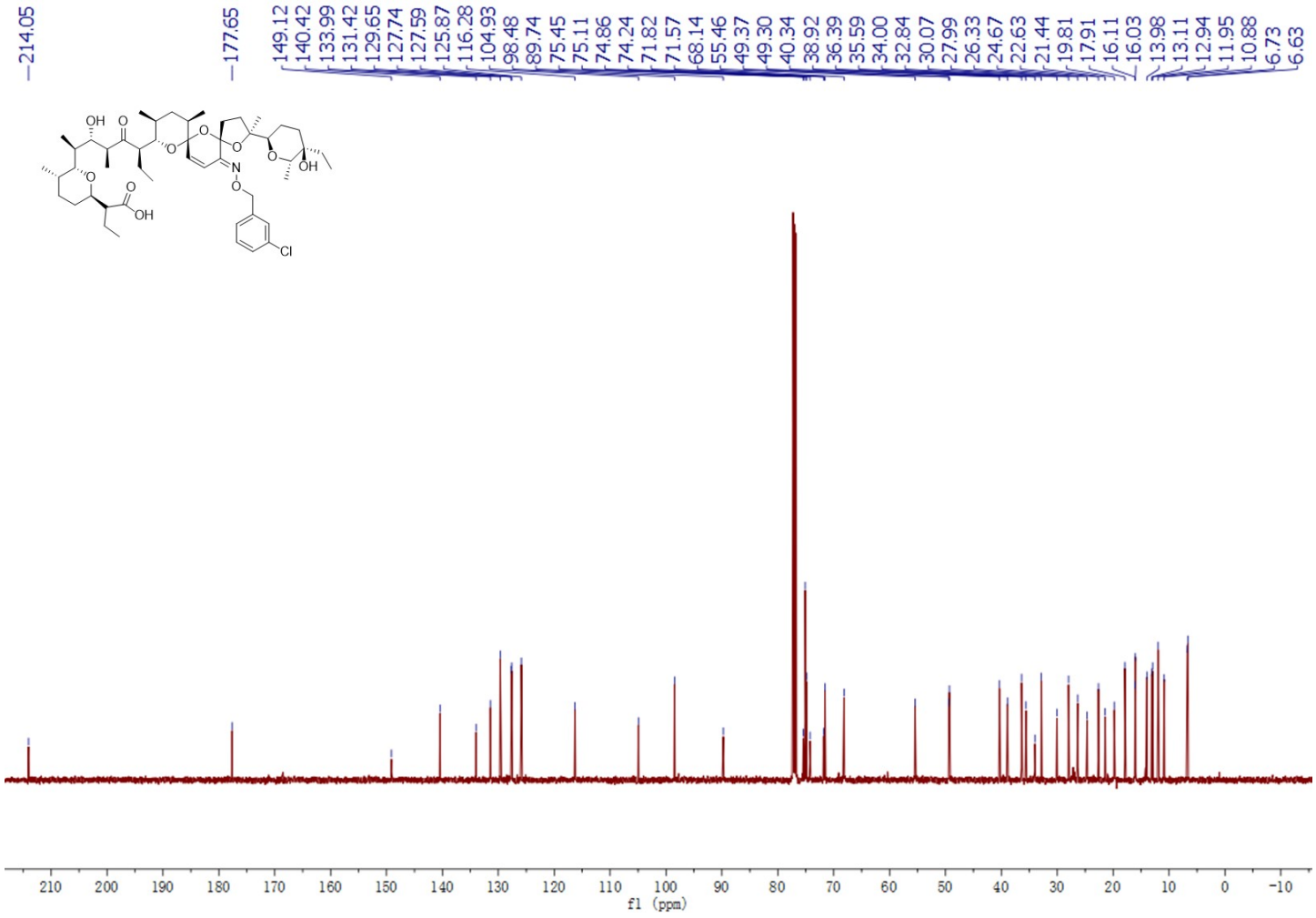
^{13}C NMR spectrum of compound **11** in $CDCl_3$



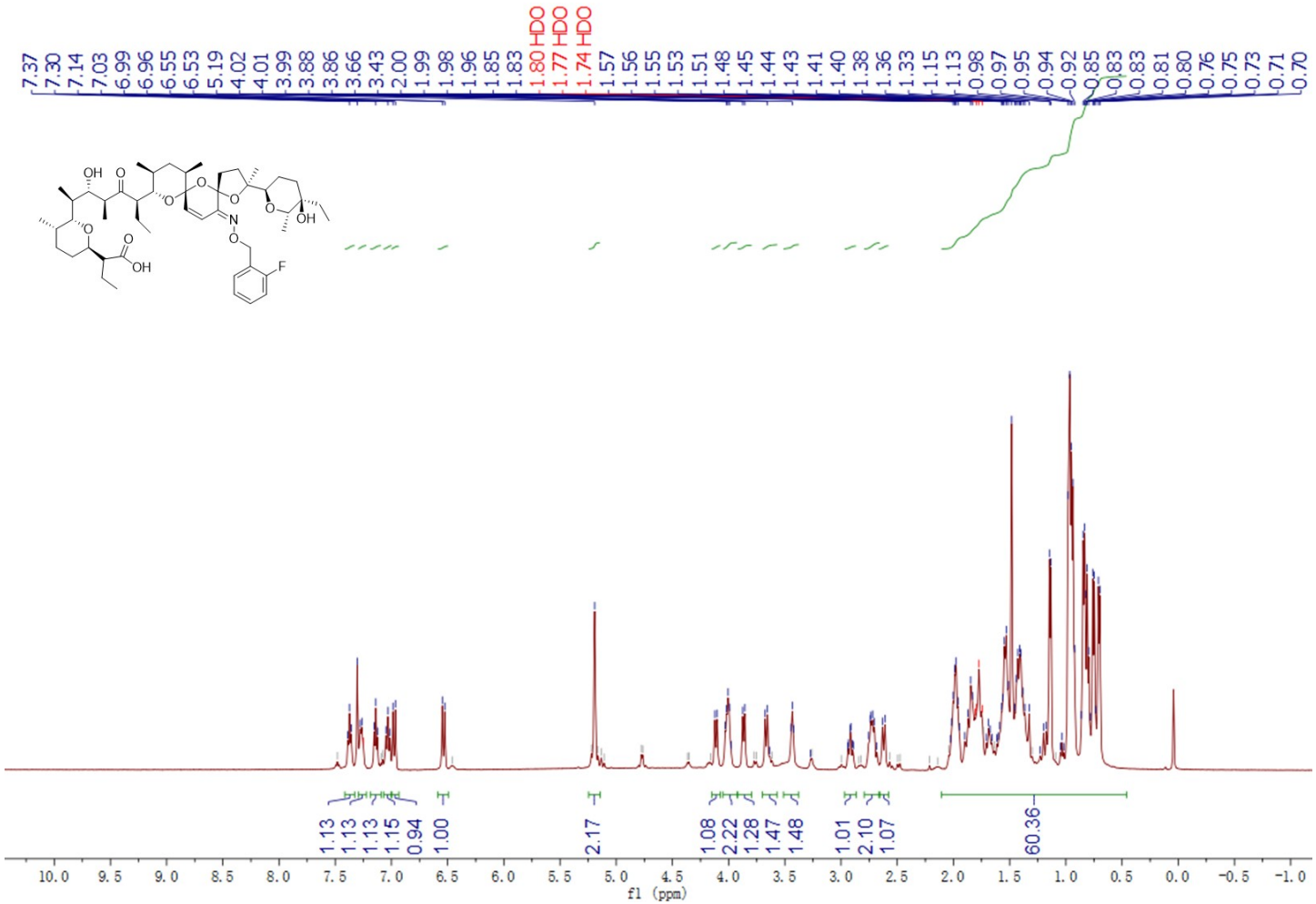
^{1}H NMR spectrum of compound **12** in $CDCl_3$



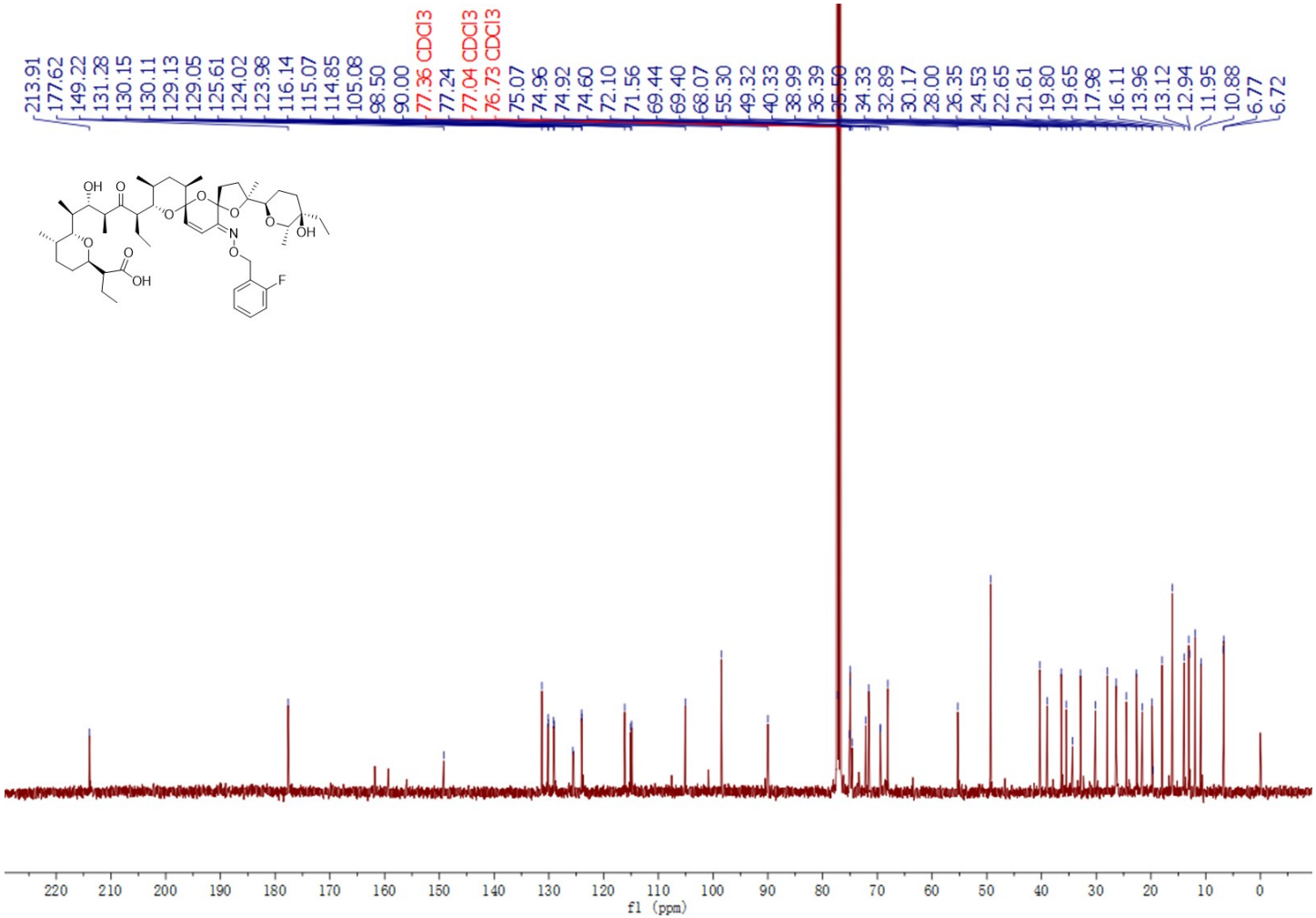
^{13}C NMR spectrum of compound **12** in $CDCl_3$



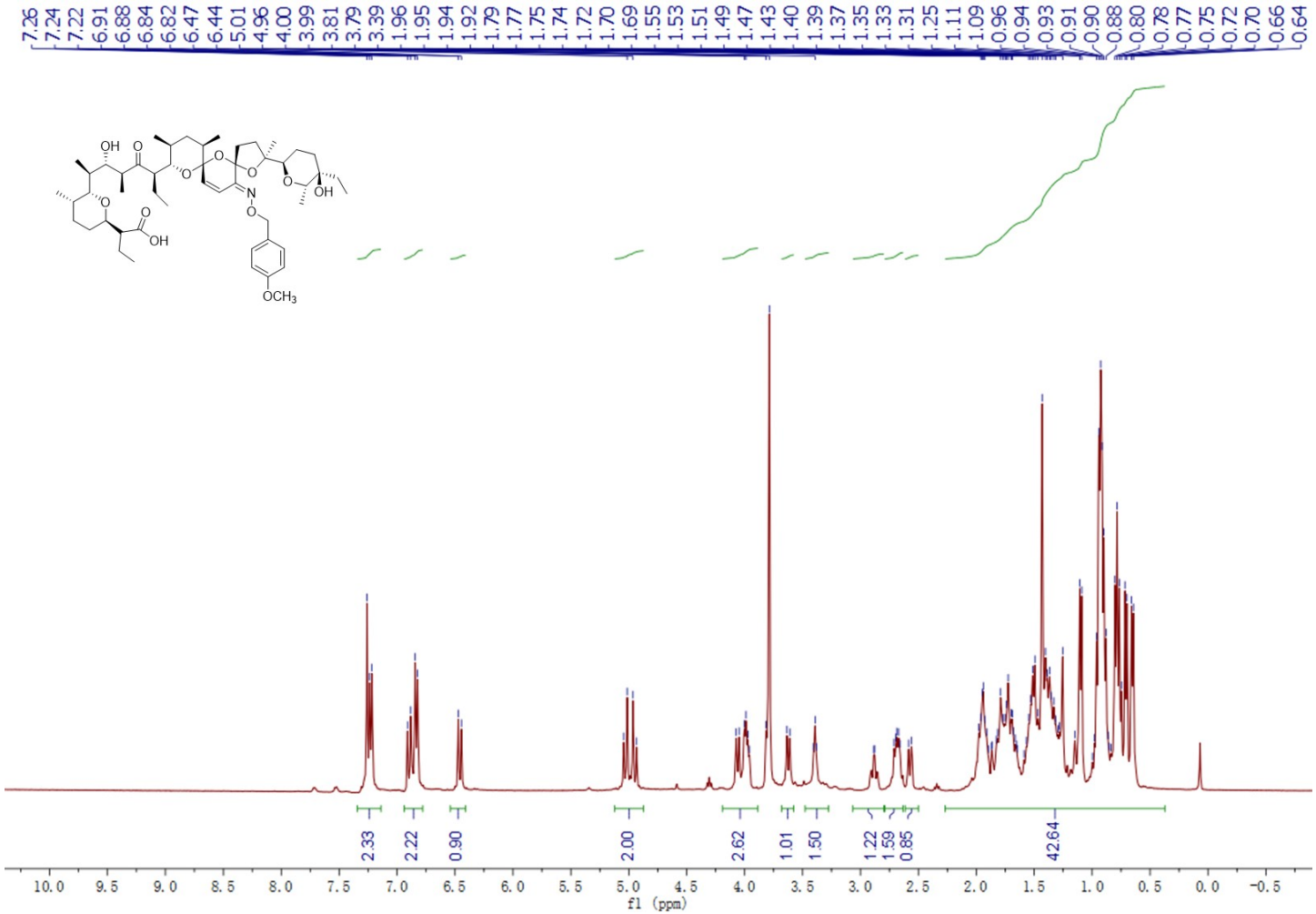
^{1}H NMR spectrum of compound **13** in $CDCl_3$



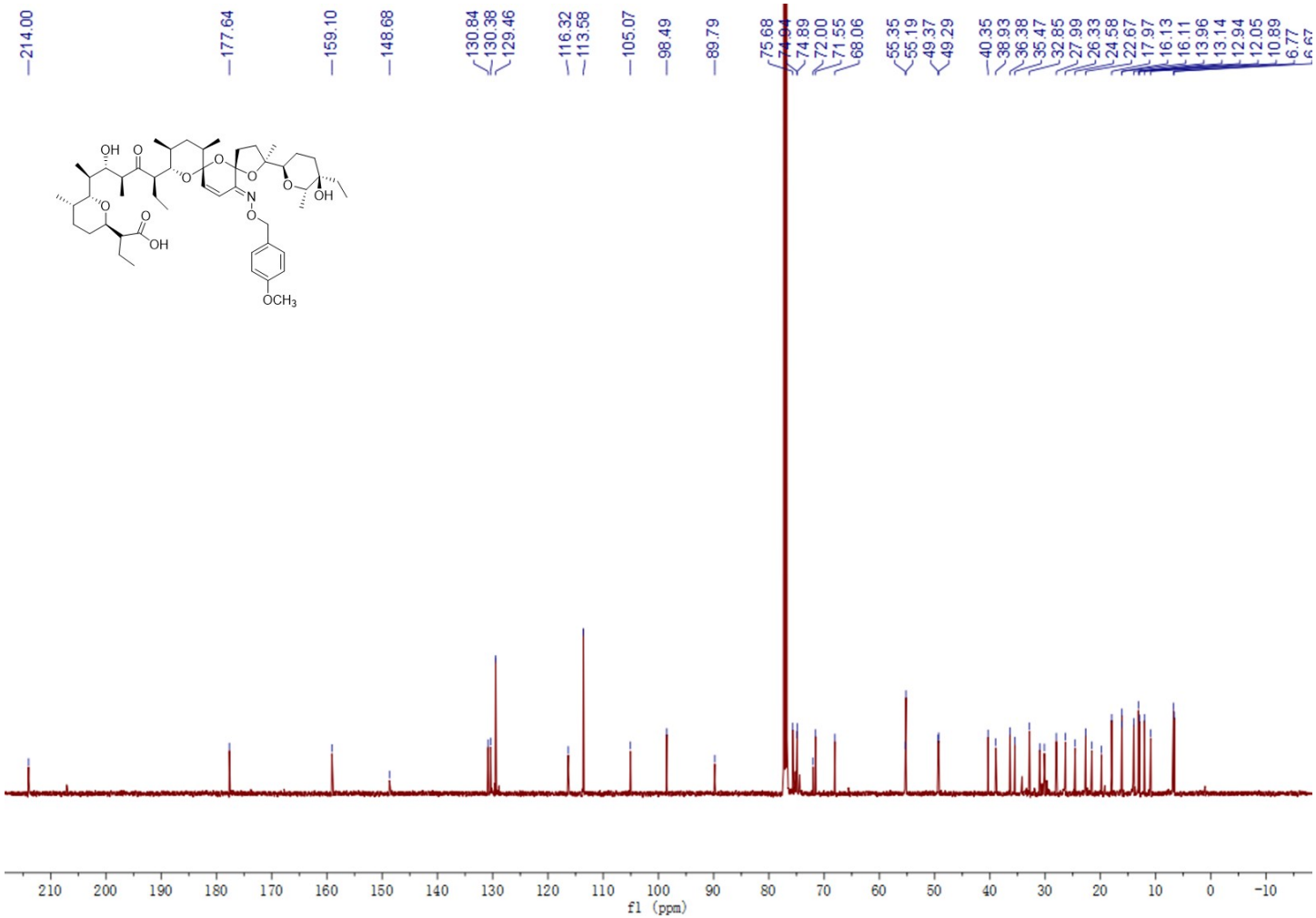
^{13}C NMR spectrum of compound **13** in $CDCl_3$



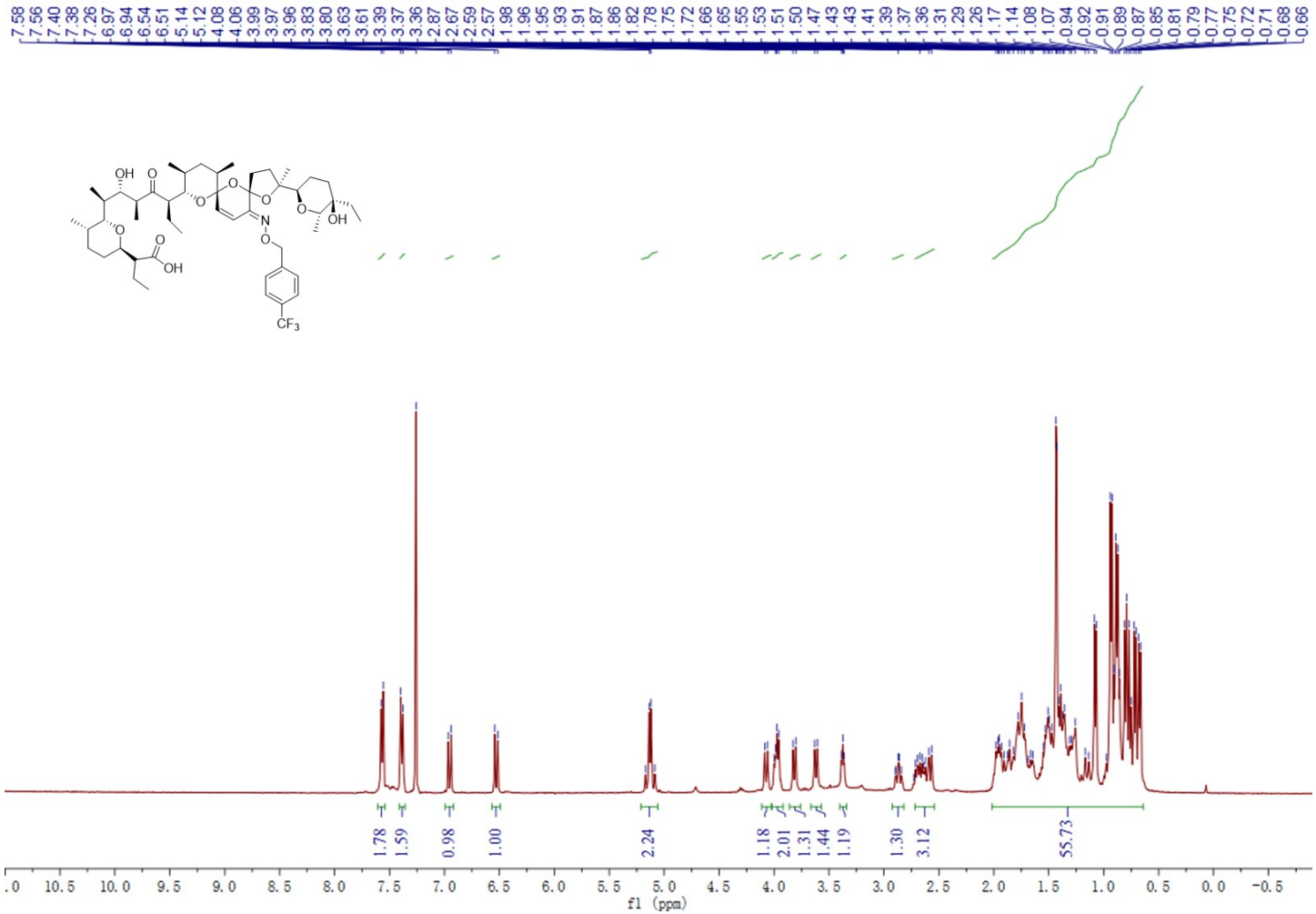
^{1}H NMR spectrum of compound **14** in $CDCl_3$



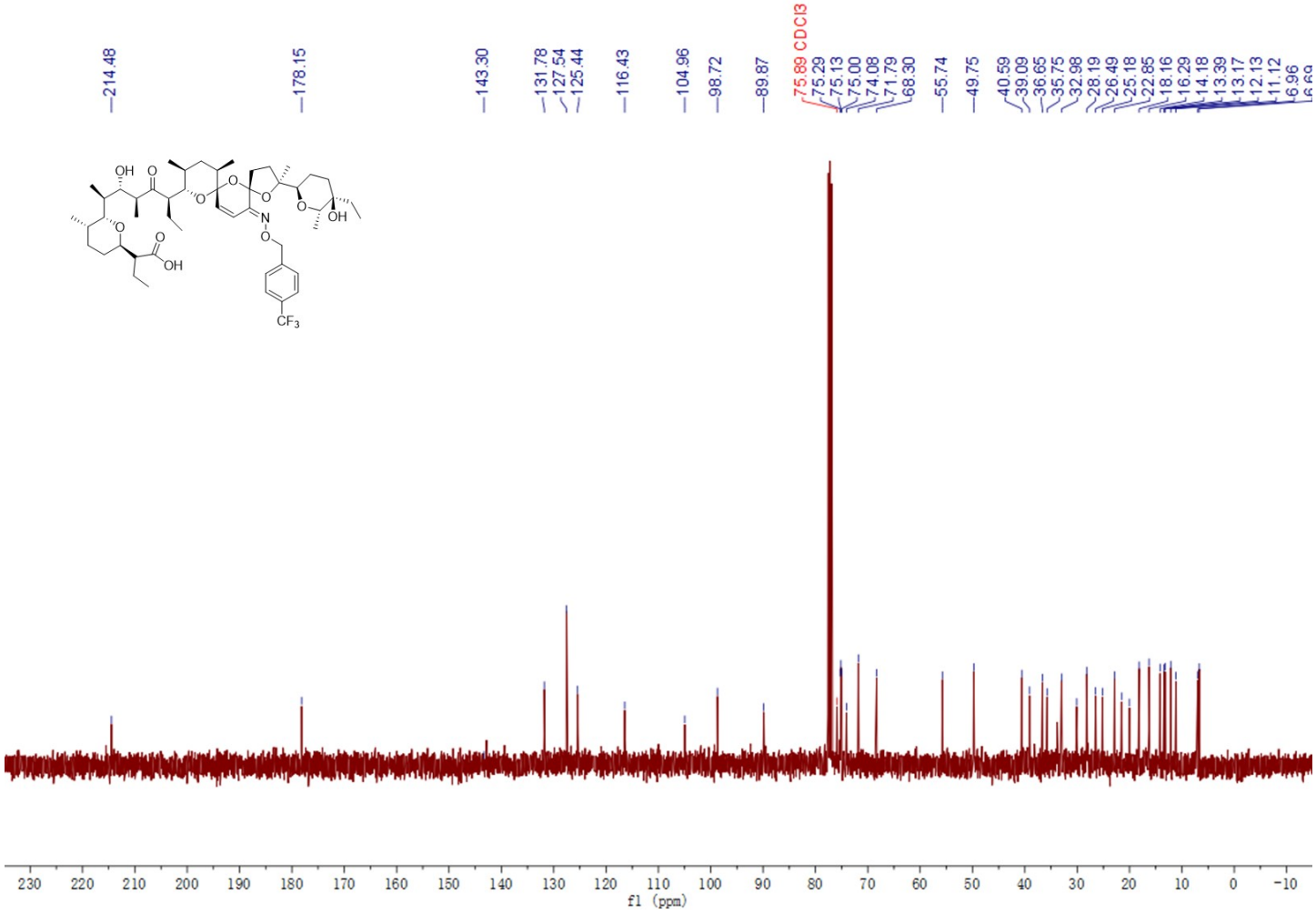
^{13}C NMR spectrum of compound **14** in $CDCl_3$



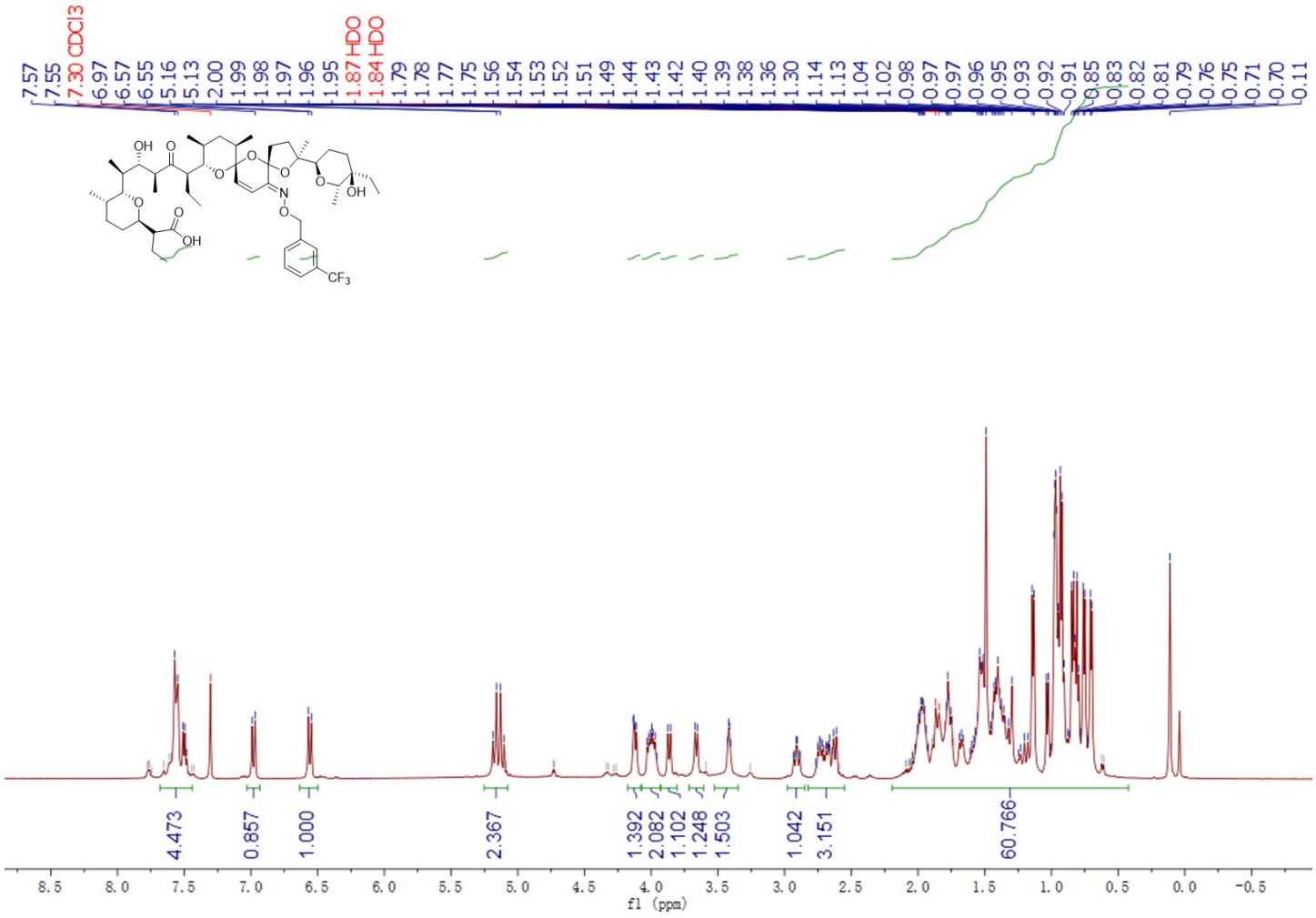
^{1}H NMR spectrum of compound **15** in $CDCl_3$



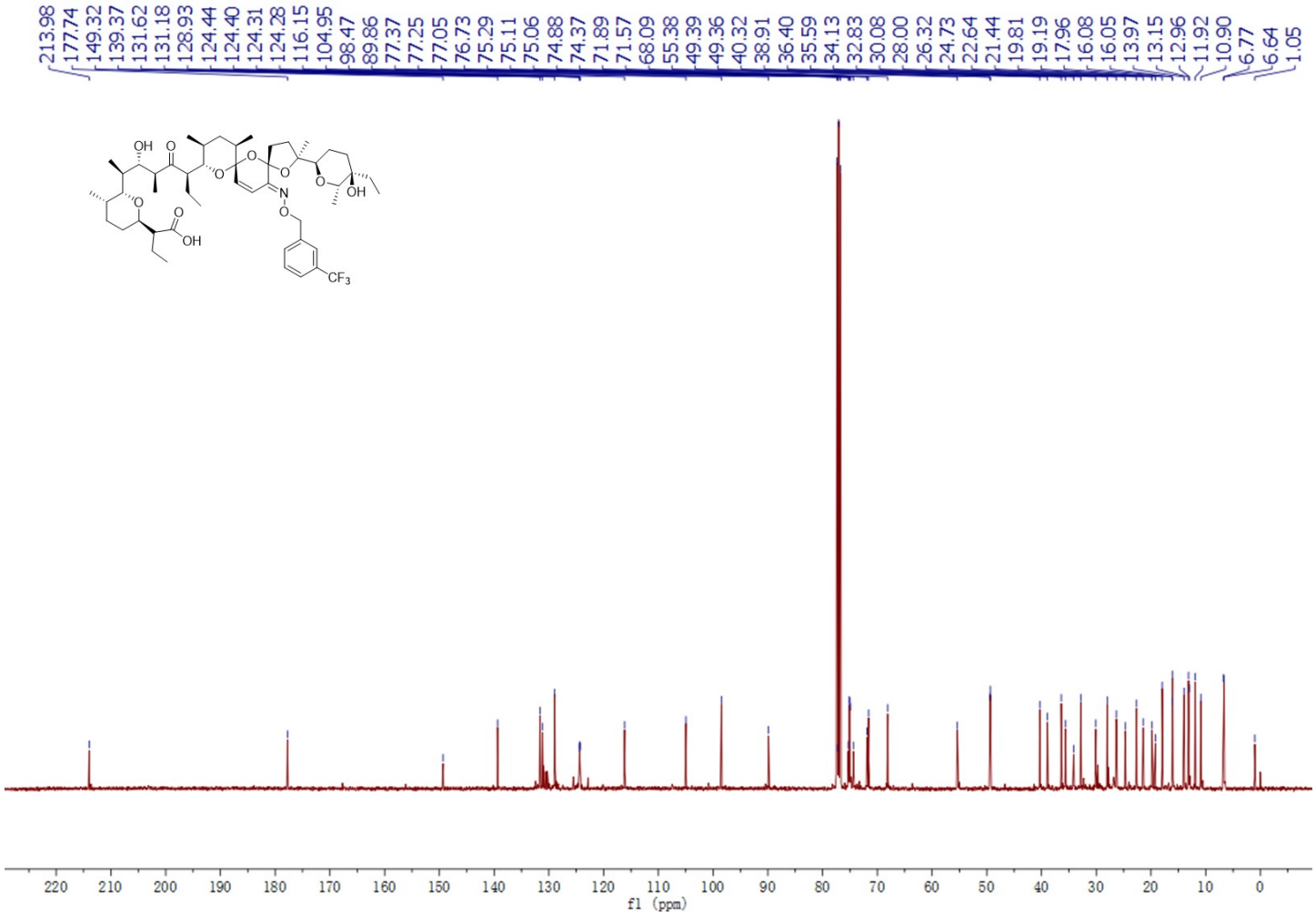
^{13}C NMR spectrum of compound **15** in $CDCl_3$



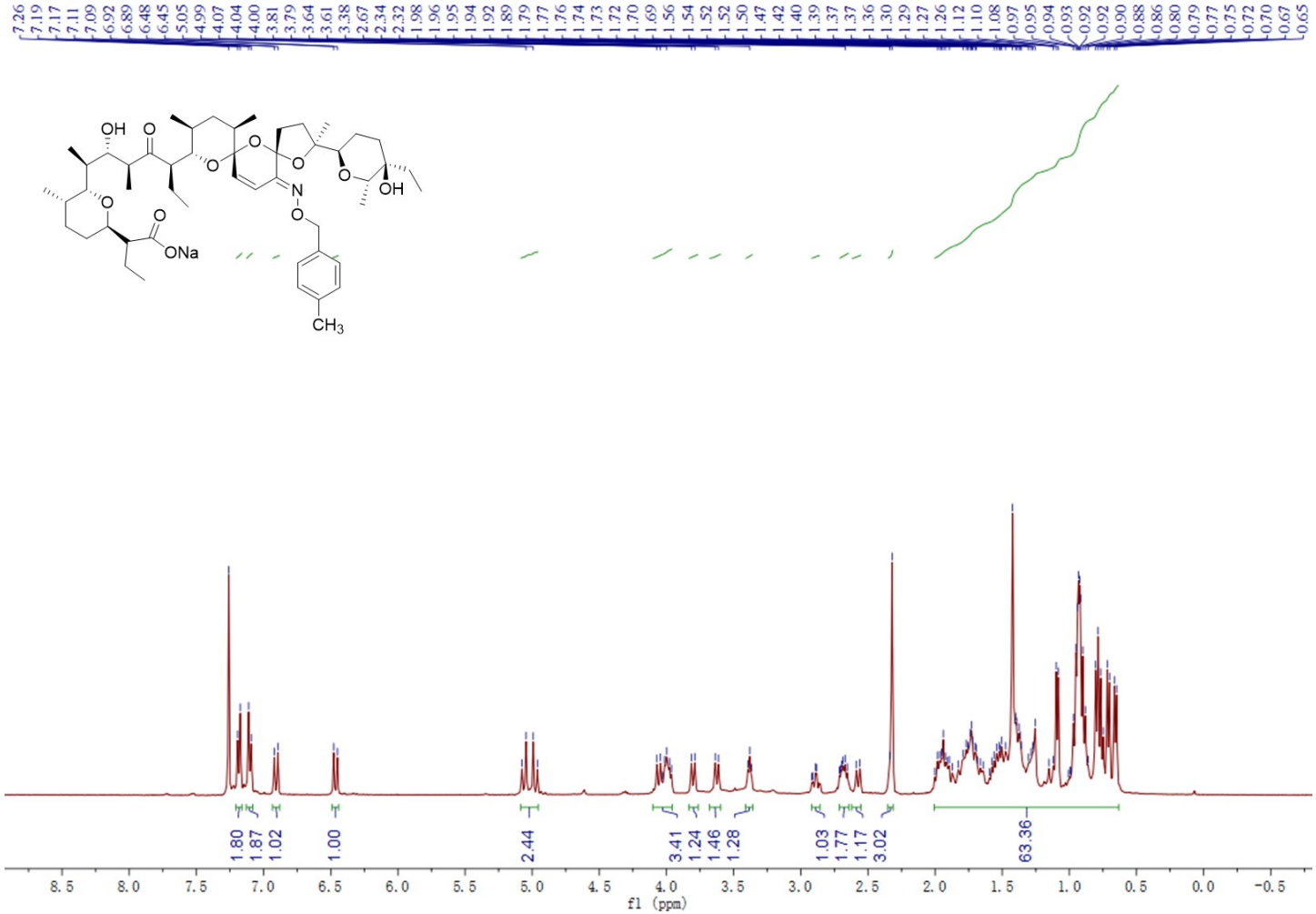
^{1}H NMR spectrum of compound **16** in $CDCl_3$



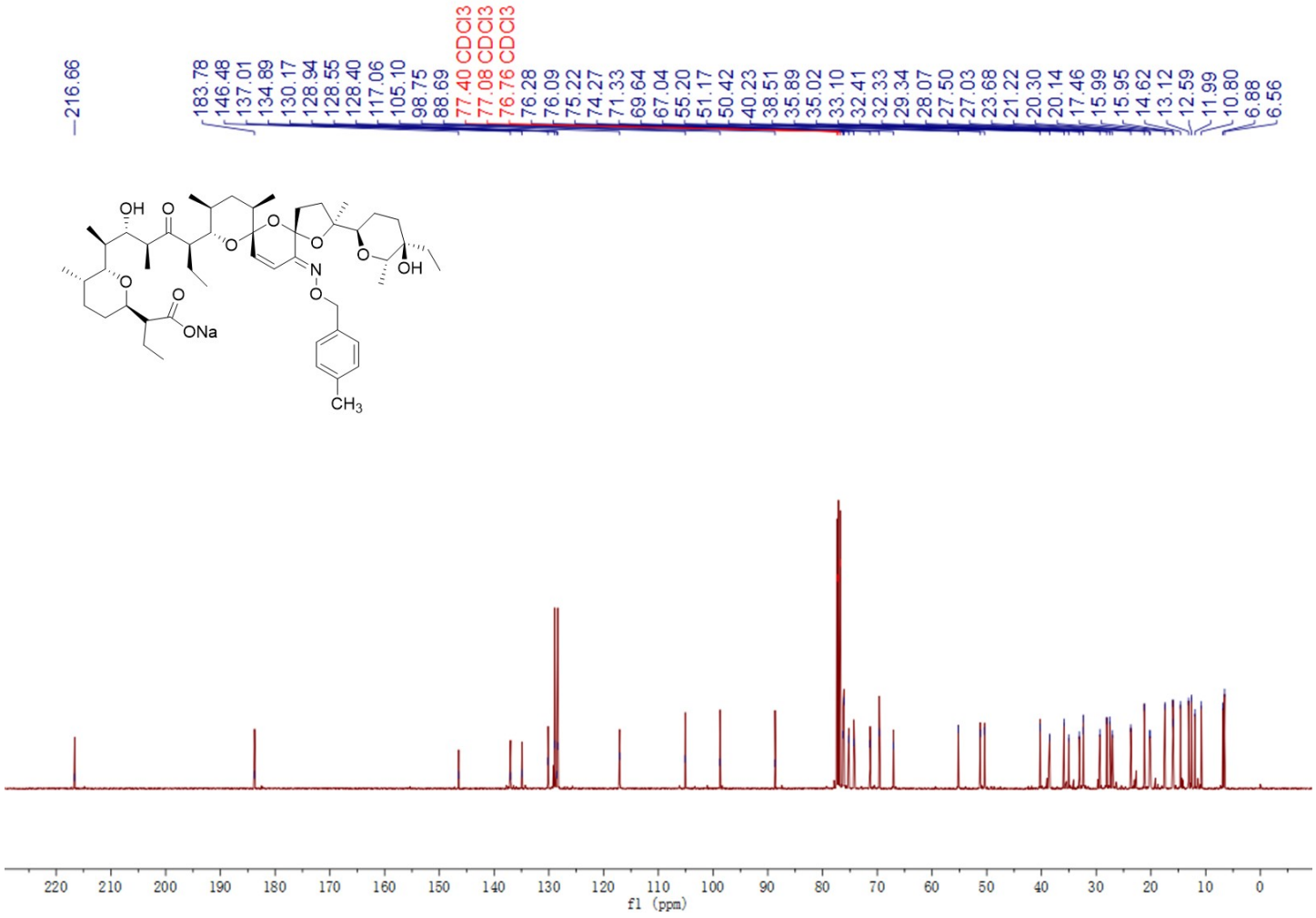
^{13}C NMR spectrum of compound **16** in $CDCl_3$



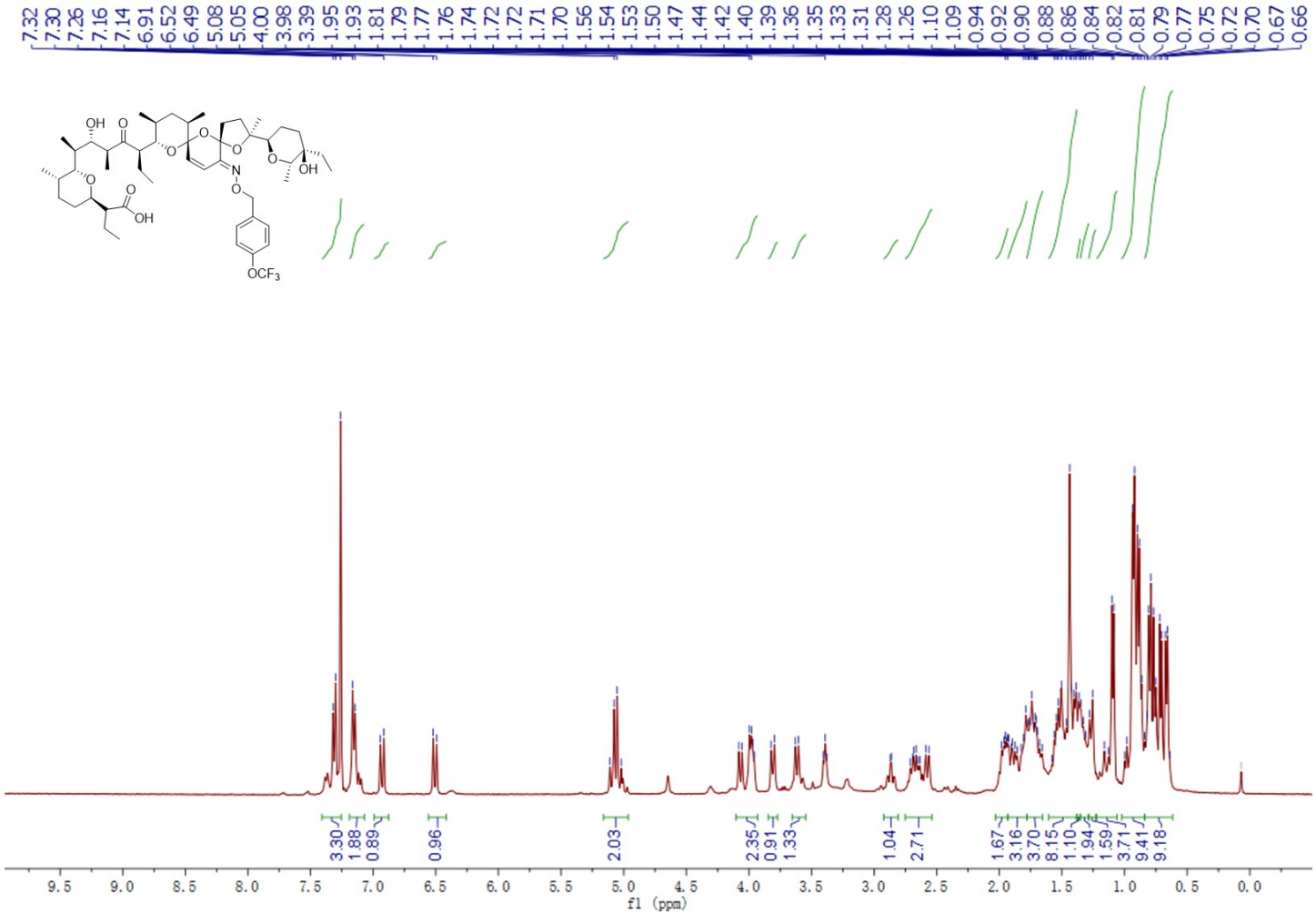
^{1}H NMR spectrum of compound **17** in $CDCl_3$



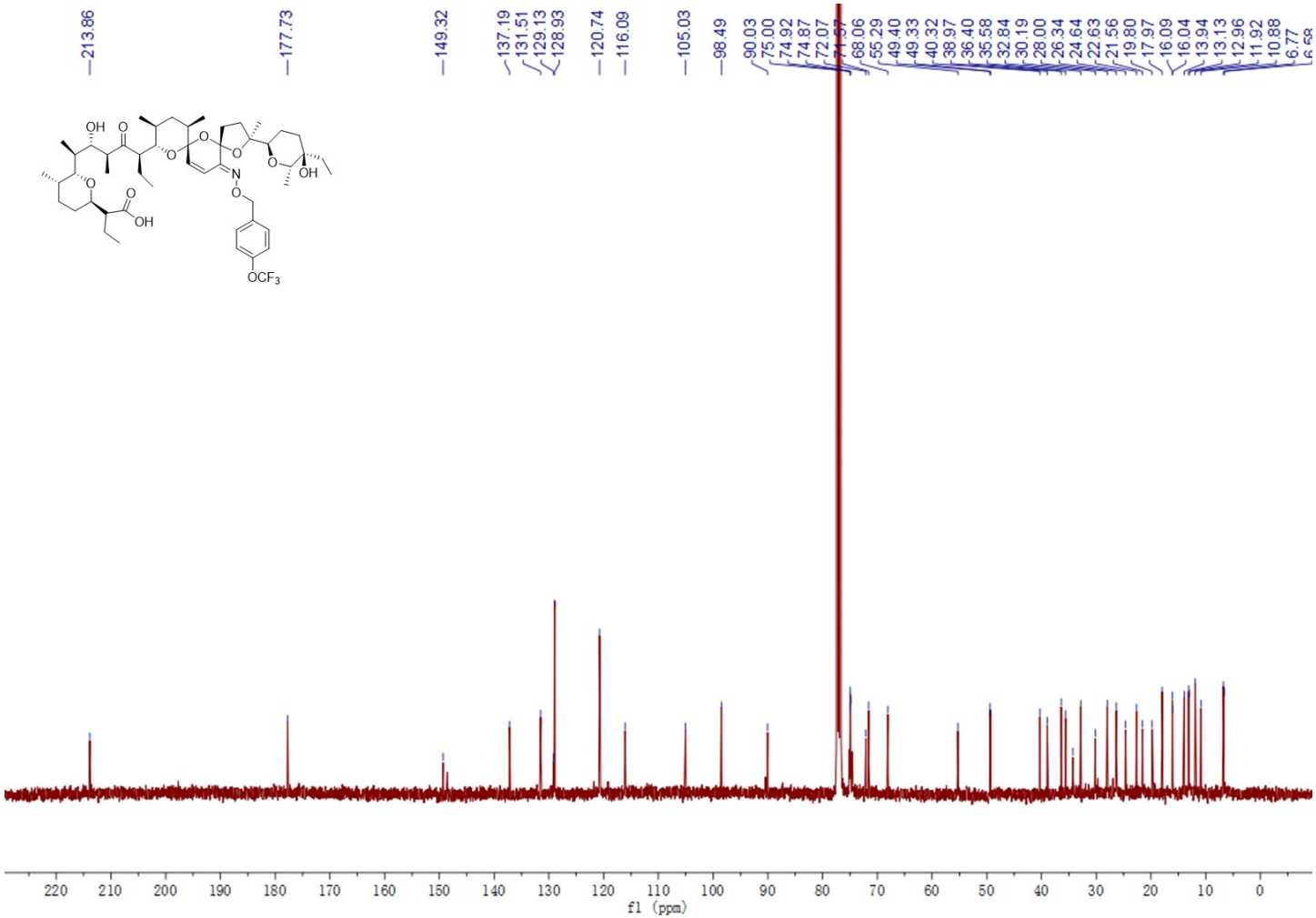
^{13}C NMR spectrum of compound **17** in $CDCl_3$



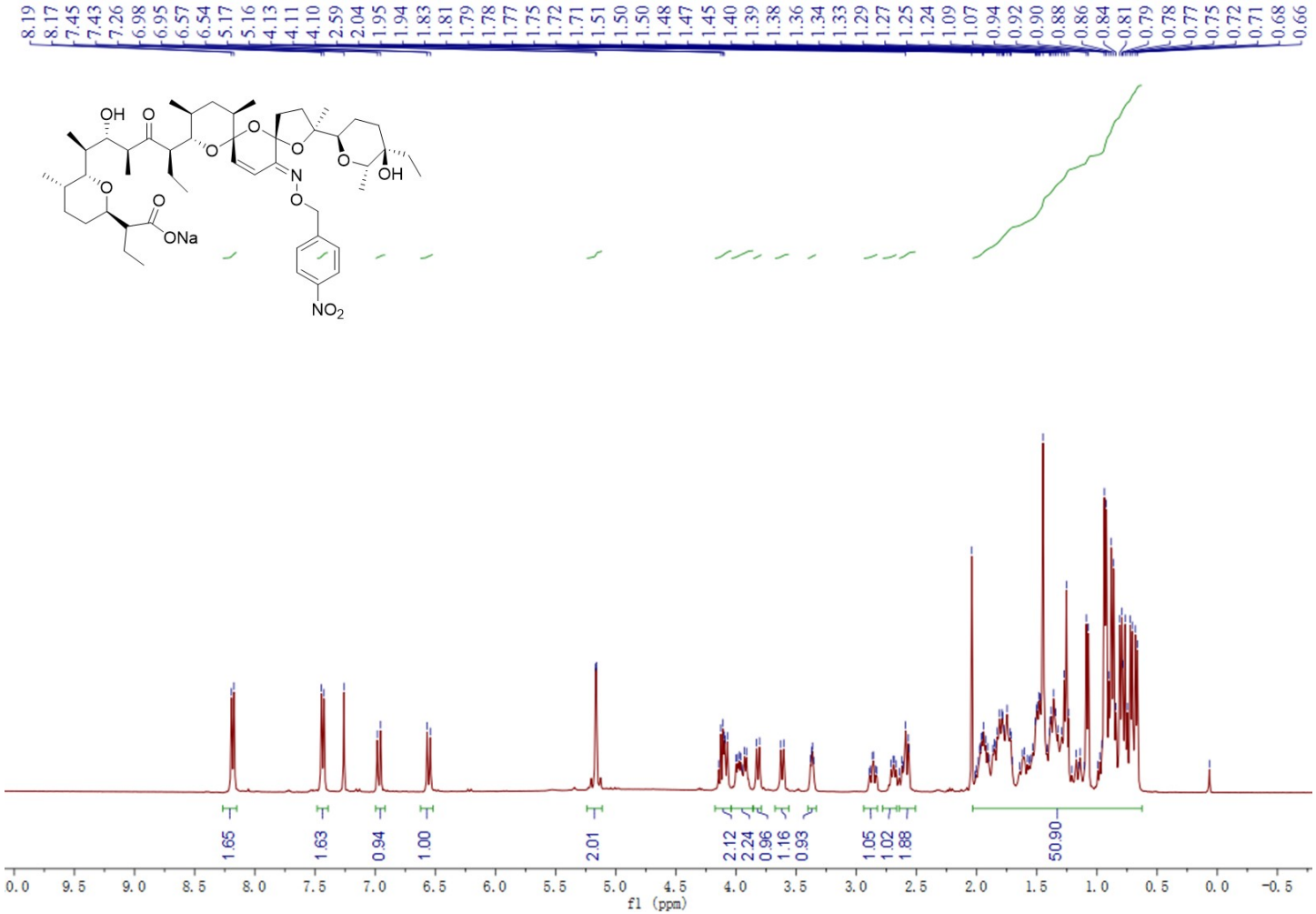
^{1}H NMR spectrum of compound **18** in $CDCl_3$



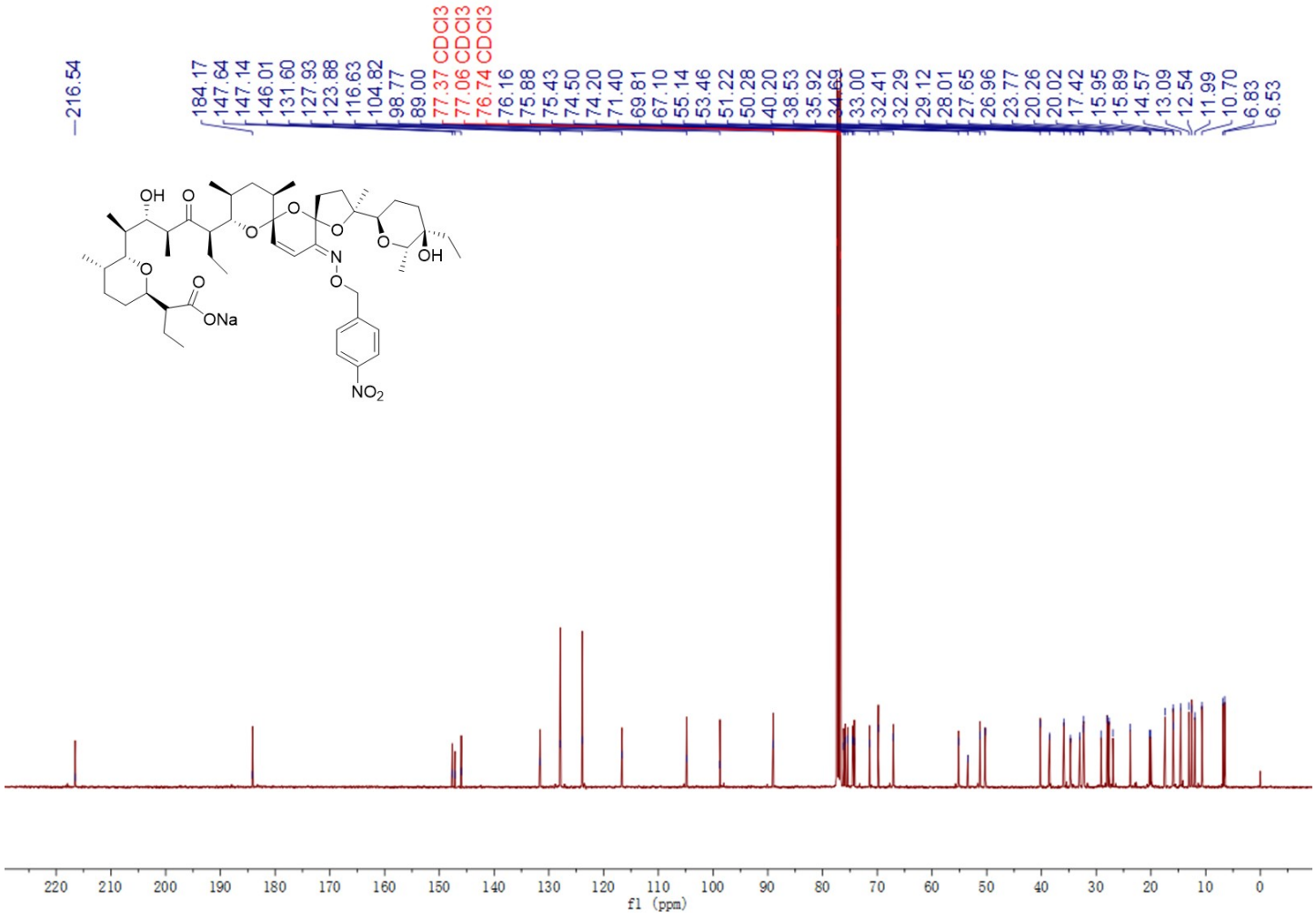
^{13}C NMR spectrum of compound **18** in $CDCl_3$



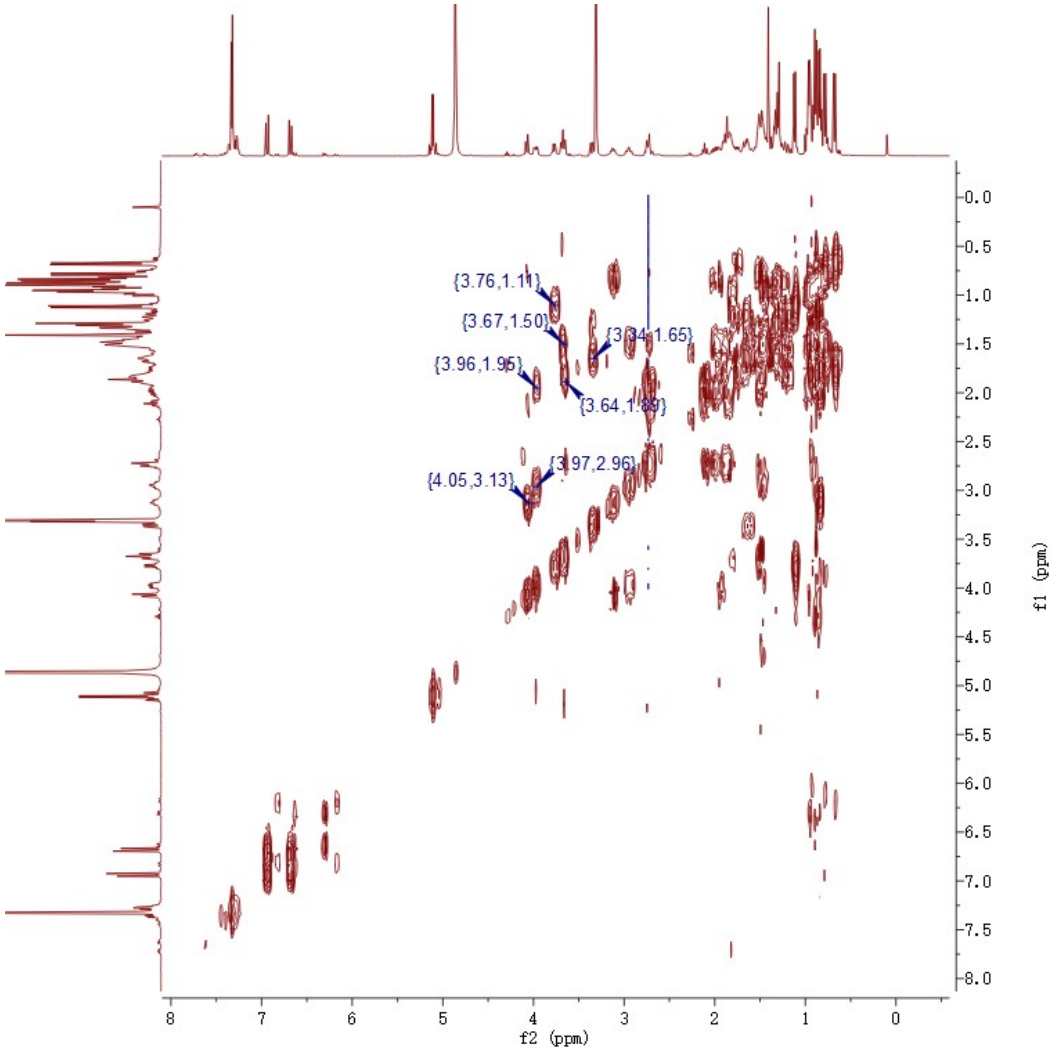
^{1}H NMR spectrum of compound **19** in $CDCl_3$



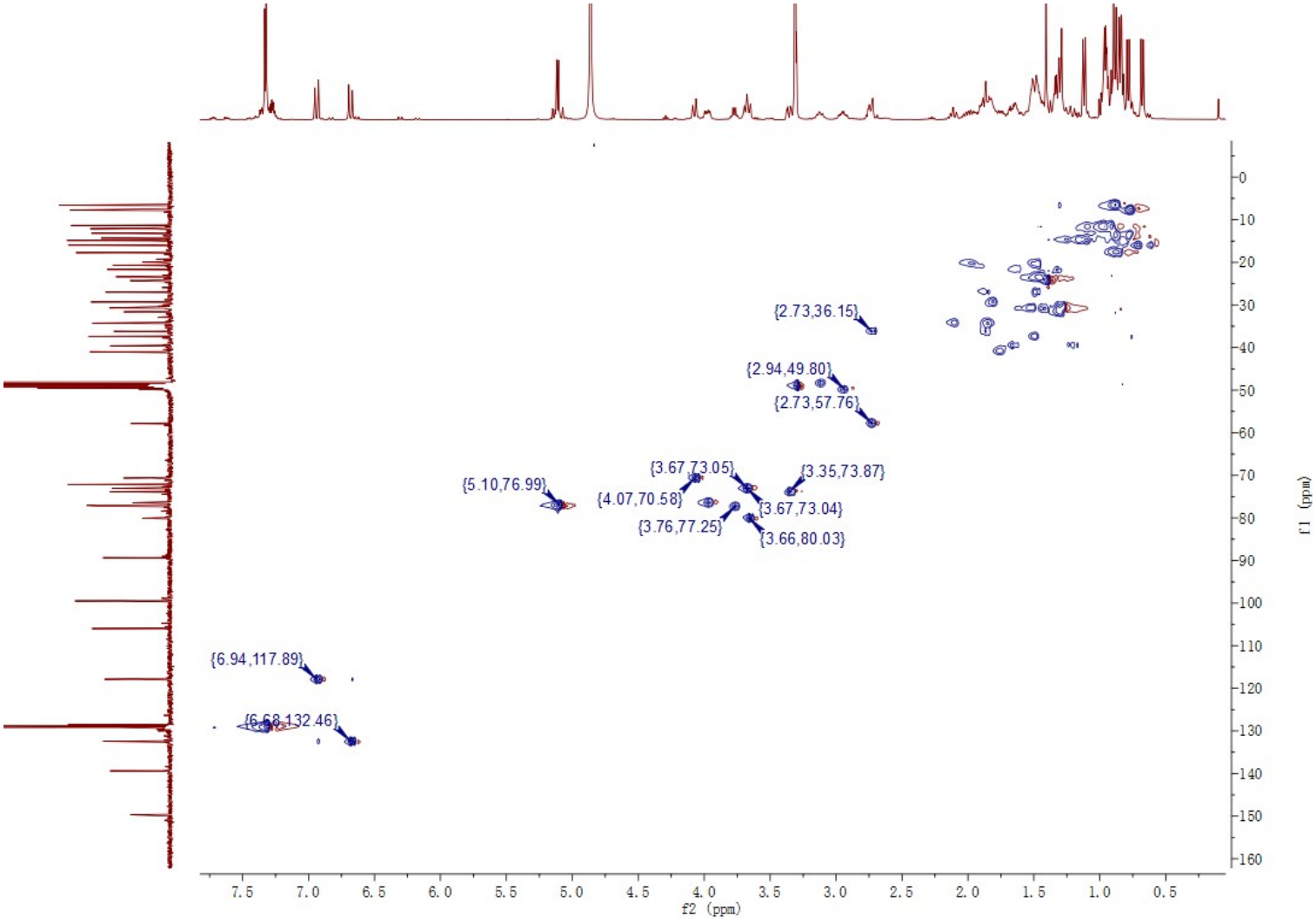
^{13}C NMR spectrum of compound **19** in $CDCl_3$



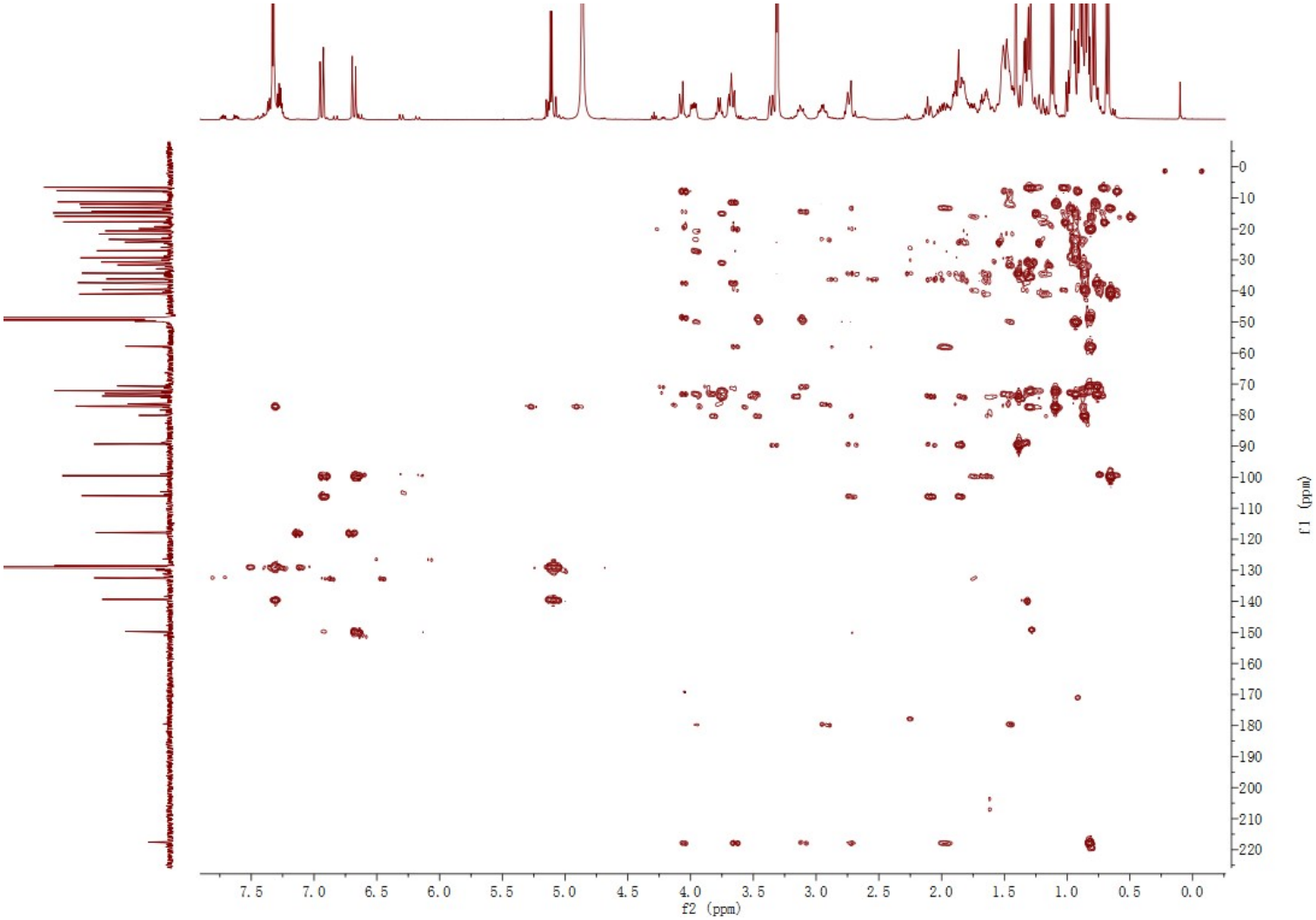
^{1}H-COSY NMR pectrum of compound **10 in CD_3OD**



^{1}H-^{13}C HSQC NMR spectrum of compound **10 in CD_3OD**



^{1}H-^{13}C HMBC spectrum of compound 10 **in CD_3OD**



IR spectra of Salinomycin (**Sal**), compound **2** and compound **6**