Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Biselyngbyolides A & C: Total Synthesis and Their Anticancer Activities

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Supporting Information

1. ^{1}H and ^{13}C-NMR Comparison of Natural Biselyngbyolides A and C with Synthetic Biselyngbyolides A and C.

Table S1: ^{1}H and ^{13}C NMR Comparison of Natural Biselyngbyolide A with Synthetic Biselyngbyolide A (4).

	^{1}H		^{13}C	
Position	Reported	Synthesized	Reported	Synthesized
	CD_3OD (400 MHz)	CD_3OD (400 MHz)	CD_3OD (100 MHz)	CD_3OD (126 MHz)
1			172.4	172.4
2	2.24 (m) 2.35 (m)	2.33 – 2.27 (m) 2.44 – 2.39 (m)	44.4	44.5
3	4.07 (m)	4.14 - 4.07 (m)	68.5	68.6
4	1.43 (m) 1.64 (m)	1.49 – 1.43 (m) 1.69 – 1.60 (m)	45.3	45.3
5	3.65 (m)	3.66 (m)	69.5	69.5
6	1.59 (m, 2H)	1.60 (m, 2H)	41.3	41.3
7	3.74 (dd, 8.0, 6.6)	3.78 (dd, *J* = 8.0, 6.3 Hz, 1H),	87.8	87.8
8			133.3	133.4
9	5.17 (brd, 9.2)	5.21 (d, *J* = 9.0 Hz, 2H)	**137.9**	**137.8**
10	2.62 (m)	2.70 – 2.61 (m, 1H)	34.1	34.1
11	1.90 (m) 2.26 (m)	1.98 – 1.87 (m) 2.32 – 2.26 (m)	42.1	42.1
12	5.47 (m)	5.56 – 5.46 (m)	133.5	133.5
13	5.97 (m)	6.06 – 5.93 (m)	132.1	132.1
14	6.00 (m)	6.09 – 5.95 (m)	**137.9**	**135.2**
15	5.49 (m)	5.54 – 5.47 (m)	127.4	127.4
16	2.24 (m) 2.34 (m)	2.39 – 2.24 (m, 2H)	39.8	39.7
17	5.56 (td, 9.2, 3.4)	5.62 – 5.55 (m)	71.6	71.7
18	5.16 (brd, 9.2)	5.21 (d, *J* = 9.0 Hz, 2H)	124.9	124.9
19			140.3	140.3
20	2.73 (dd, 15.0, 6.5) 2.94 (brdd, 15.0, 6.5)	2.98 (dd, *J* = 14.5, 6.8 Hz, 1H) 2.77 (dd, *J* = 14.5, 6.2 Hz, 1H)	36.7	36.6
21	5.35 (m)	5.39 - 5.35 (m)	129.4	129.4
22	5.39 (m)	5.48 – 5.38 (m)	127.7	127.7
23	1.64 (m, 3H)	1.67 (d, *J* = 1.5 Hz, 3H)	18.0	18.0
24	3.16 (s, 3H)	3.16 (s, 3H)	55.8	55.7
25	1.51 (d, 1.2, 3H)	1.54 (d, *J* = 1.4 Hz, 3H)	10.2	10.2
26	1.02 (d, 6.6, 3H)	1.06 (d, *J* = 6.7 Hz, 3H)	22.4	22.4
27	1.68 (d, 1.4, 3H)	1.61 (d, *J* = 1.5 Hz, 3H)	23.5	23.5

References for Reported NMR of Biselyngbyolide A (4): (a) M. Morita, O. Ohno and K. Suenaga, *Chem. Lett*. 2012, **41**, 165-167. (b) Y. Tanabe, E. Sato, N. Nakajima, A. Ohkubo, O. Ohno and K. Suenaga, *Org. Lett*. 2014, **16**, 2858-2861.

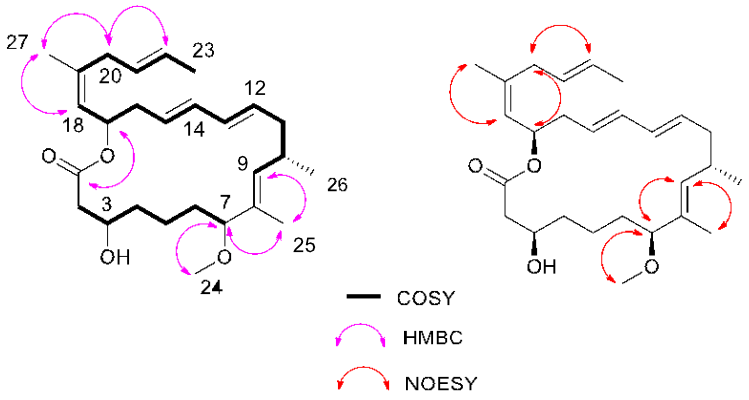
Table S2: ^{1}H and ^{13}C NMR Comparison of Natural Biselyngbyolide C with Synthetic Biselyngbyolide C (6).

^{1}H			^{13}C	
Position	Reported	Synthesized	Reported	Synthesized
	C_6D_6 (400 MHz)	C_6D_6 (500 MHz)	C_6D_6 (100 MHz)	C_6D_6 (126 MHz)
1			172.0	172.00
2	2.14 (dd, 2.8, 14.8) 2.36 (dd, 8.8, 14.8)	2.14 (dd, 10, 15) 2.35 (dd, 5, 15)	42.7	42.8
3	3.88 (m)	3.89 (m)	68.8	68.8
4	1.20 (m) 1.53 (m)	1.19 (m) 1.53 (m)	**22.5**	**36.2**
5	**1.26 (m)** **1.60 (m)**	**1.32 – 1.13 (m, 2H)**	**36.2**	**22.6**
6	1.61 (m, 2H)	1.60 (m)	32.2	32.3
7	3.41 (dd, 5.6, 8.4)	3.40 (dd, *J* = 8.4, 6.3 Hz, 1H)	88.0	88.1
8			133.7	133.8
9	4.9 (dq, 10.0, 1.2)	4.90 (dt, *J* = 9.8, 1.5 Hz, 1H)	135.4	135.5
10	2.42 (m)	2.46 – 2.38 (m, 1H)	33.0	33.2
11	2.13 (brd, 15.2) 1.75 (m)	2.15 – 2.10 (m) 1.80 – 1.72 (m)	41.4	41.5
12	5.34 (ddd, 4.0, 9.6, 15.2)	5.38 – 5.32 (m)	133.3	133.3
13	5.95 (dd, 10.8, 15.2)	5.98 – 5.88 (m)	130.9	130.9
14	5.98 (dd, 10.8, 15.2)	6.05 – 5.95 (m)	134.5	134.5
15	5.38 (m)	5.41 – 5.36 (m)	**128.6**	**126.7**
16	2.27 (m) 2.28 (m)	2.30 – 2.20 (m)	39.9	39.9
17	5.88 (dt, 9.2, 4.0)	5.92 – 5.83 (m)	70.1	70.1
18	5.27 (dq, 9.2, 1.2)	5.26 (dd, *J* = 9.0, 1.6 Hz, 1H)	124.6	124.6
19			139.6	139.7
20	2.78 (dd, 4.0, 14.8) 2.98 (dd, 4.0, 14.8)	2.80 – 2.75 (m) 2.46 – 2.38 (m)	35.8	35.9
21	5.39 (m)	5.42 – 5.37 (m)	**129.1**	**128.6**
22	5.42 (m)	5.44 – 5.39 (m)	**128.4**	**127.2**
23	1.56 (d, 1.2, 3H)	1.56 (d, *J* = 1.4 Hz, 3H)	18.0	18.0
24	3.13 (s, 3H)	3.13 (s, 3H)	55.5	55.5
25	1.57 (d, 1.2, 3H)	1.57 (d, *J* = 1.2 Hz, 3H)	10.1	10.1
26	0.92 (d, 6.8, 3H)	0.92 (d, *J* = 6.7 Hz, 3H)	22.4	22.4
27	1.62 (d, 1.2, 3H)	1.62 (d, *J* = 1.3 Hz, 3H)	23.5	23.5

References for Reported NMR of Biselyngbyolide C (6): A. Watanabe, O. Ohno, M. Morita, T. Inuzuka and K. Suenaga, *Bull. Chem. Soc. Jpn.* 2015, **88**, 1256-1264.

2. Key 2D-NMR Correlations and Structure Confirmation of Synthesized Biselyngbyolide C (6).

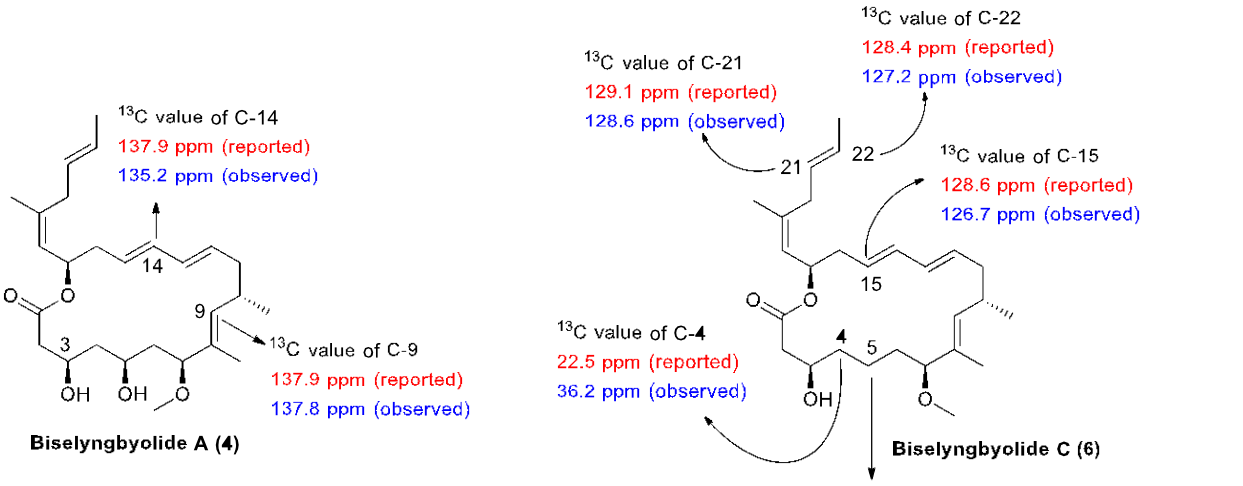
Figure S1:



2D-NMR Correlations biselyngbyolide C (6)

3. Observed NMR Misassignments for Biselyngbyolides A (4) and C (6).

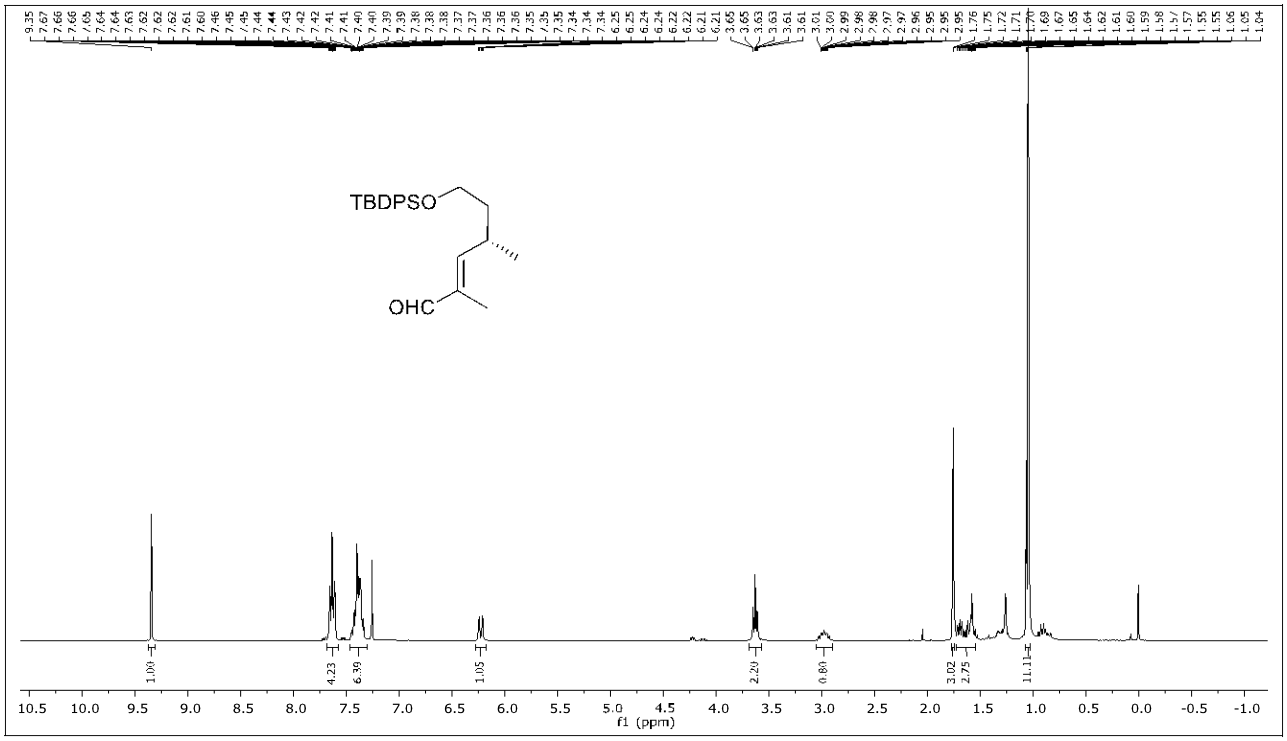
Figure S2:



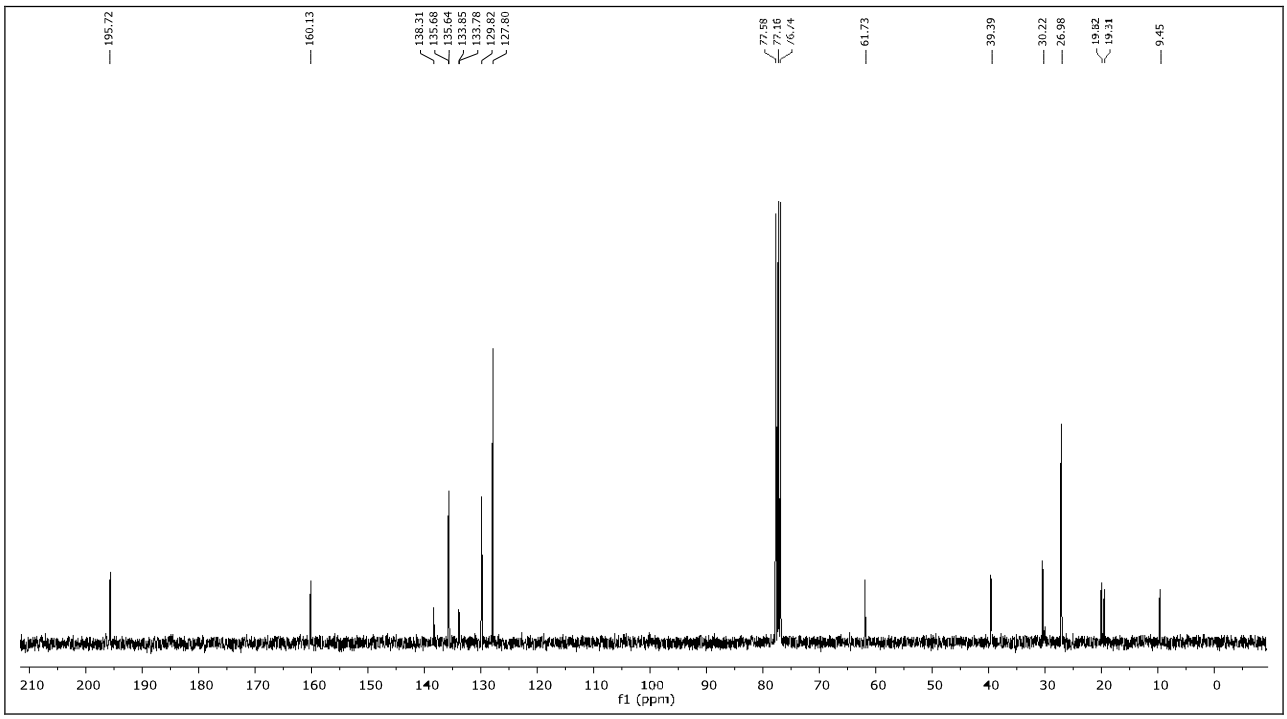
Observed NMR misassignments for biselyngbyolide A (4) biselyngbyolide C (6)

4. Copies of ^{1}H-NMR, ^{13}C-NMR, 2D-NMR and HRMS Spectra.

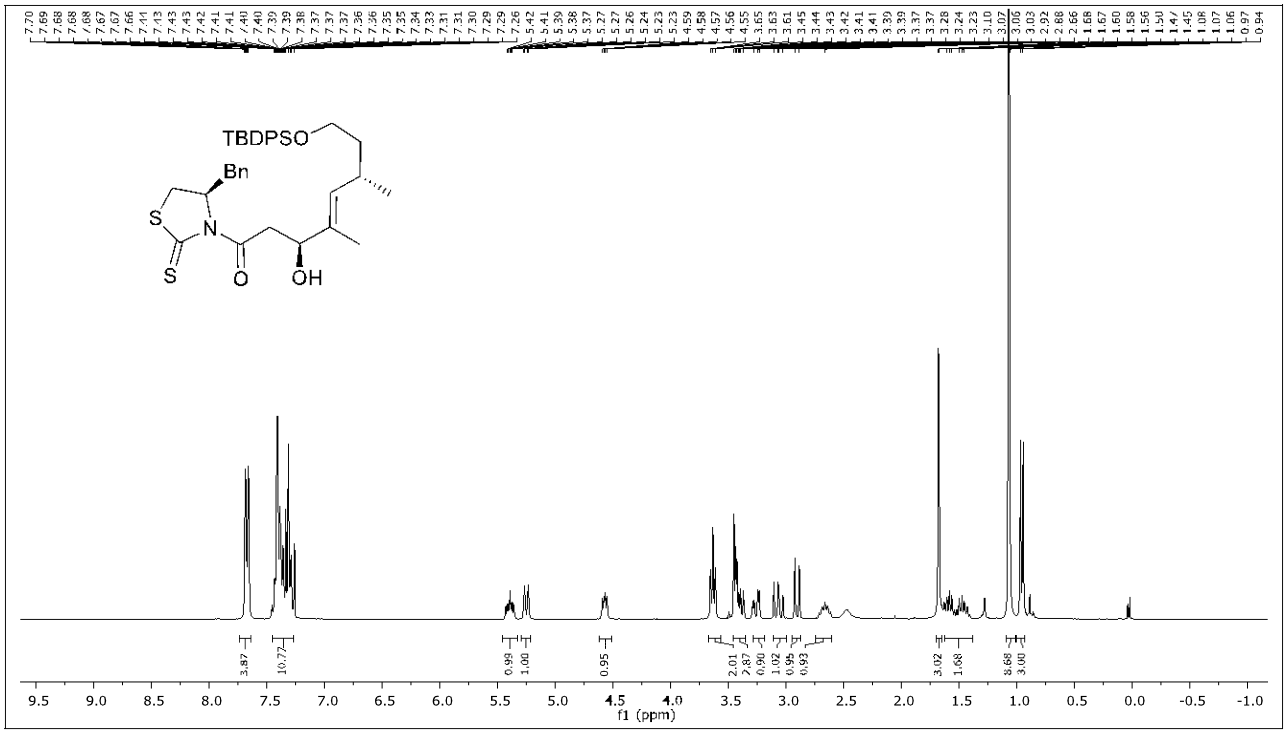
^{1}H-NMR Spectrum of Compound 17 (300 MHz, $CDCl_3$):



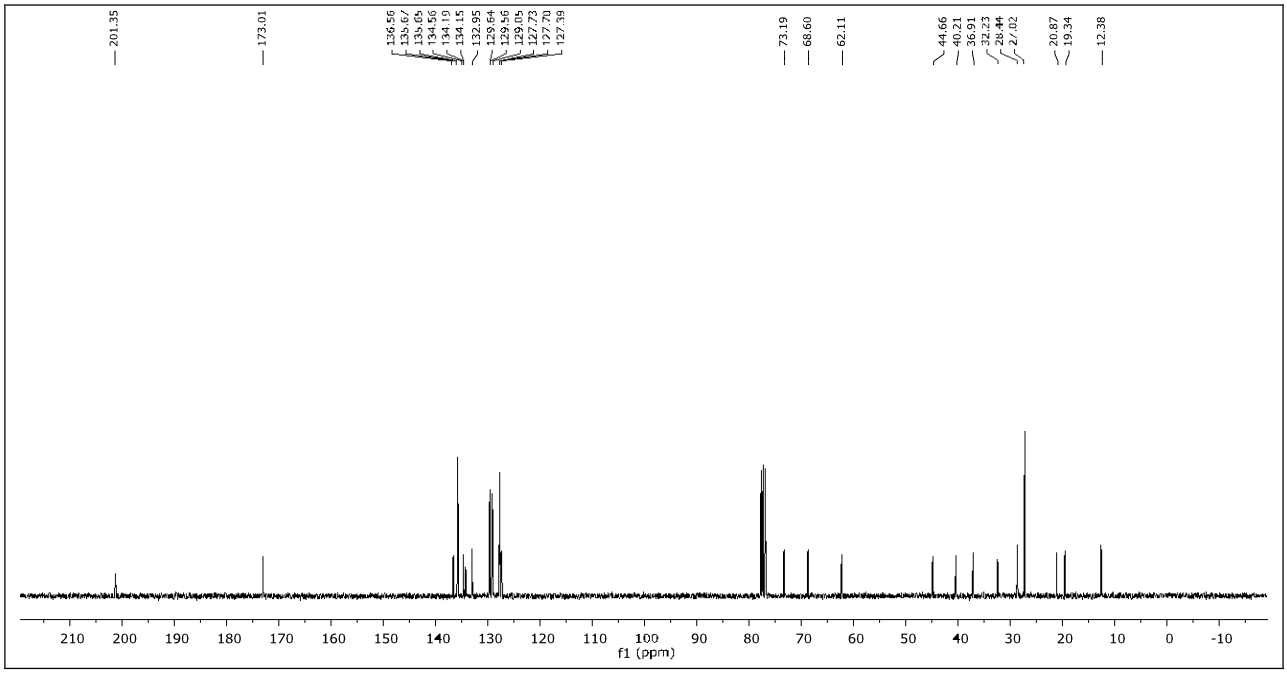
^{13}C-NMR Spectrum of Compound 17 (300 MHz, $CDCl_3$):



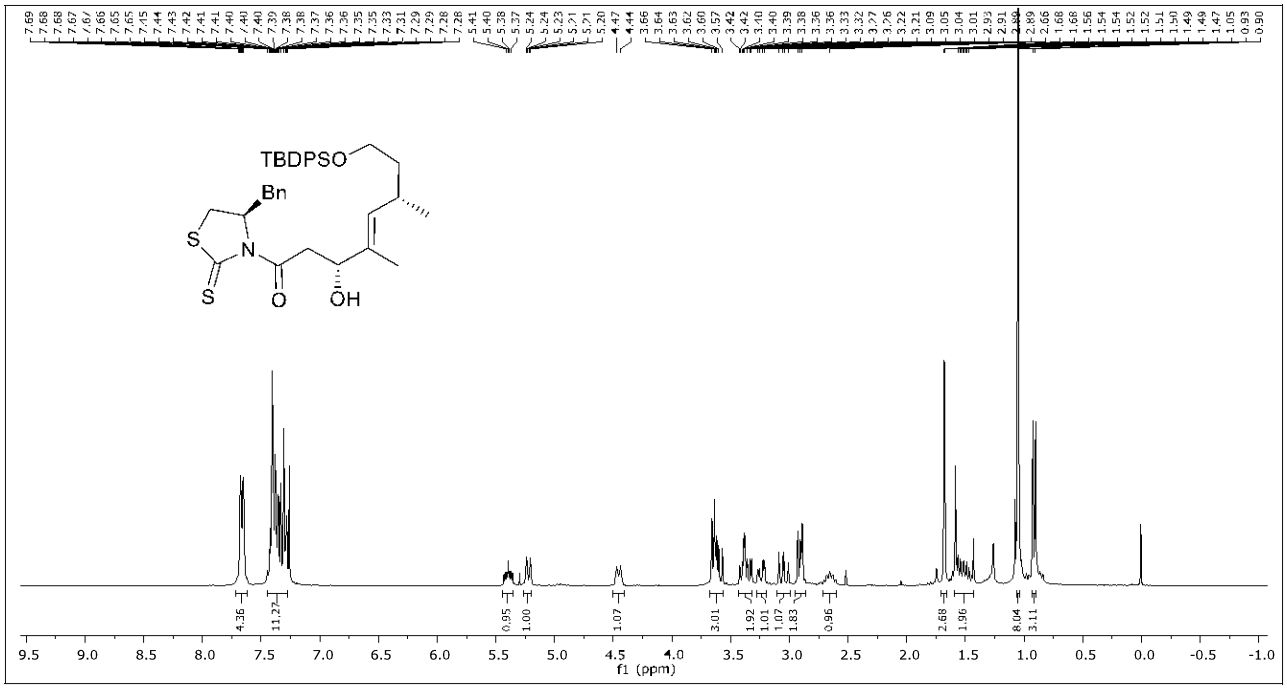
^{1}H-NMR Spectrum of Compound 19a (300 MHz, $CDCl_3$):



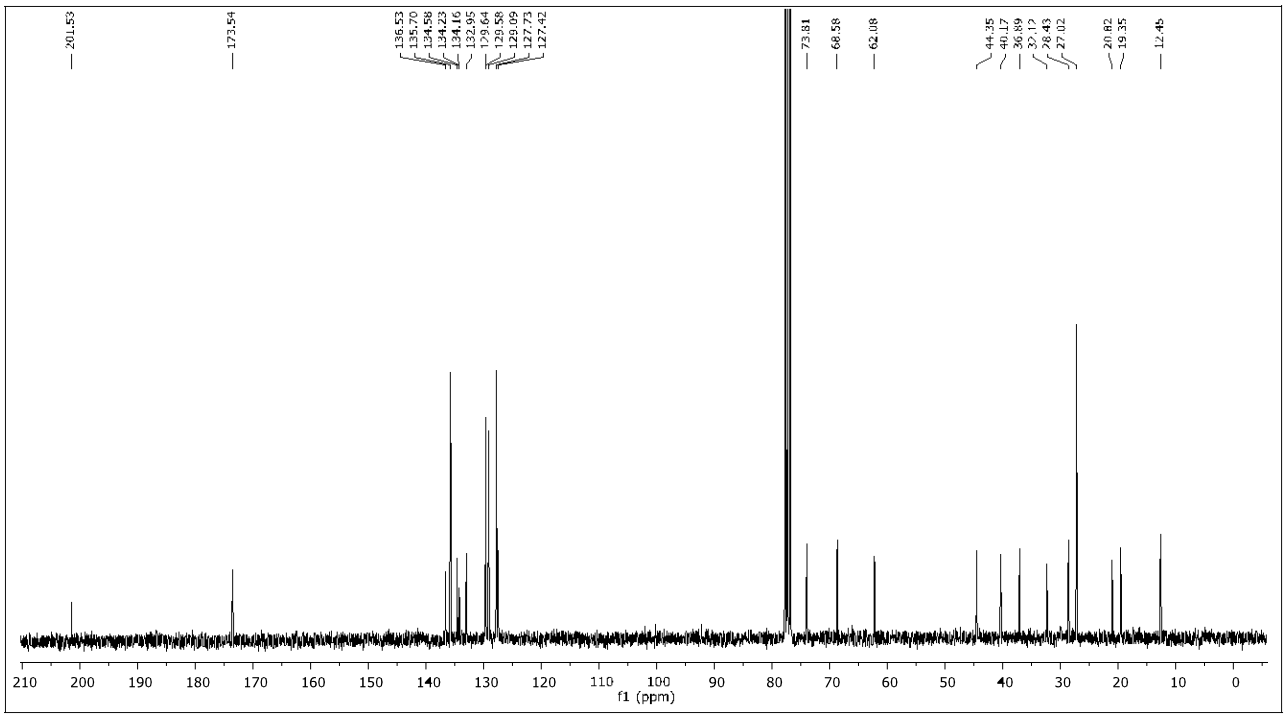
^{13}C-NMR Spectrum of Compound 19a (300 MHz, $CDCl_3$):



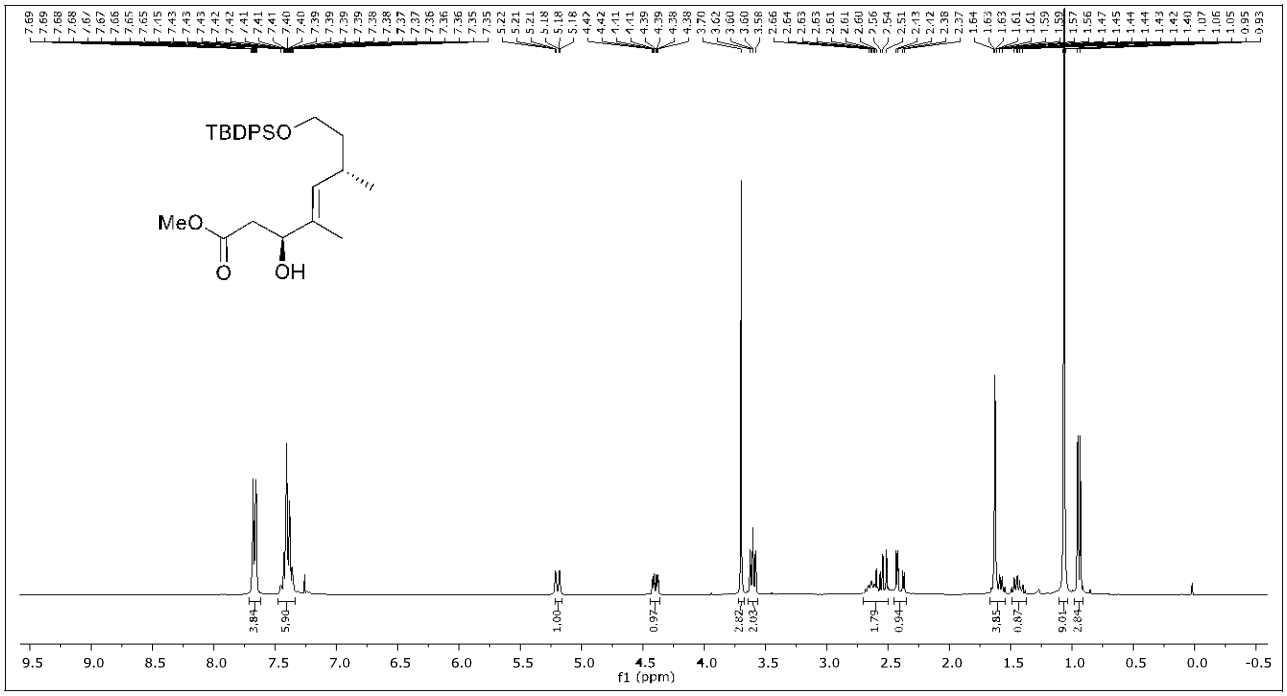
^{1}H-NMR Spectrum of Compound 19b (300 MHz, $CDCl_3$):



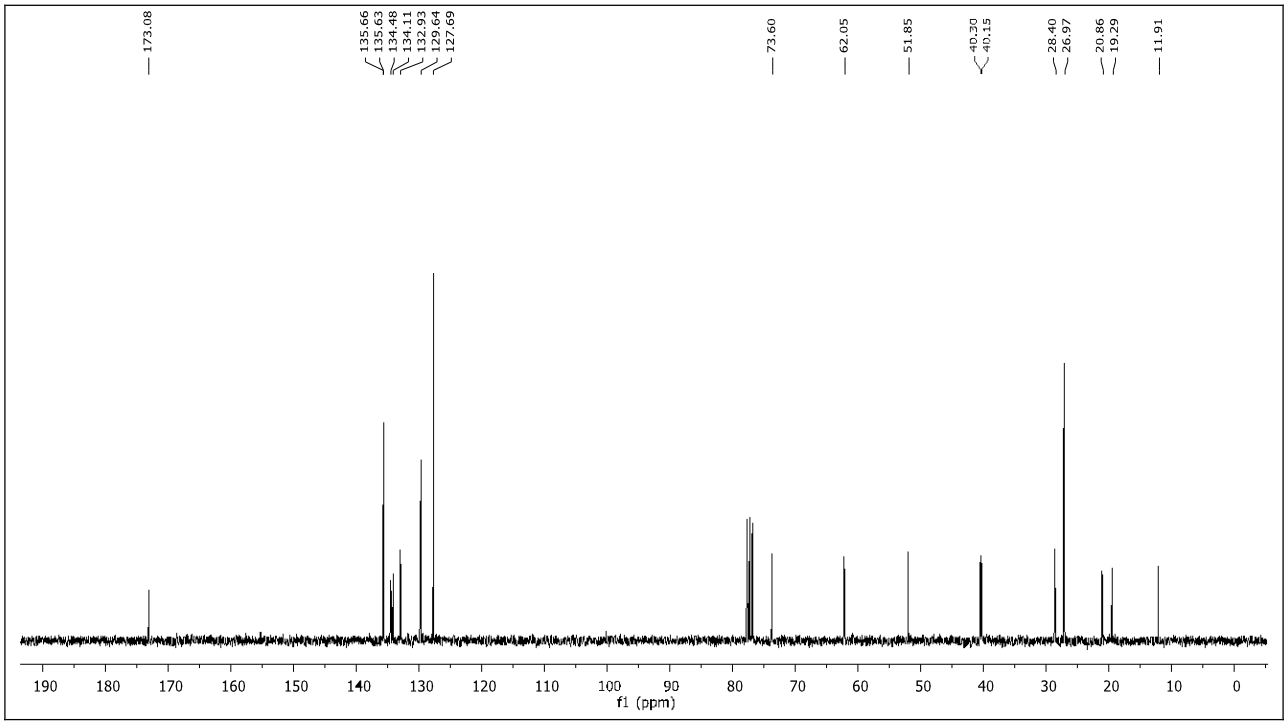
^{13}C-NMR Spectrum of Compound 19b (300 MHz, $CDCl_3$):



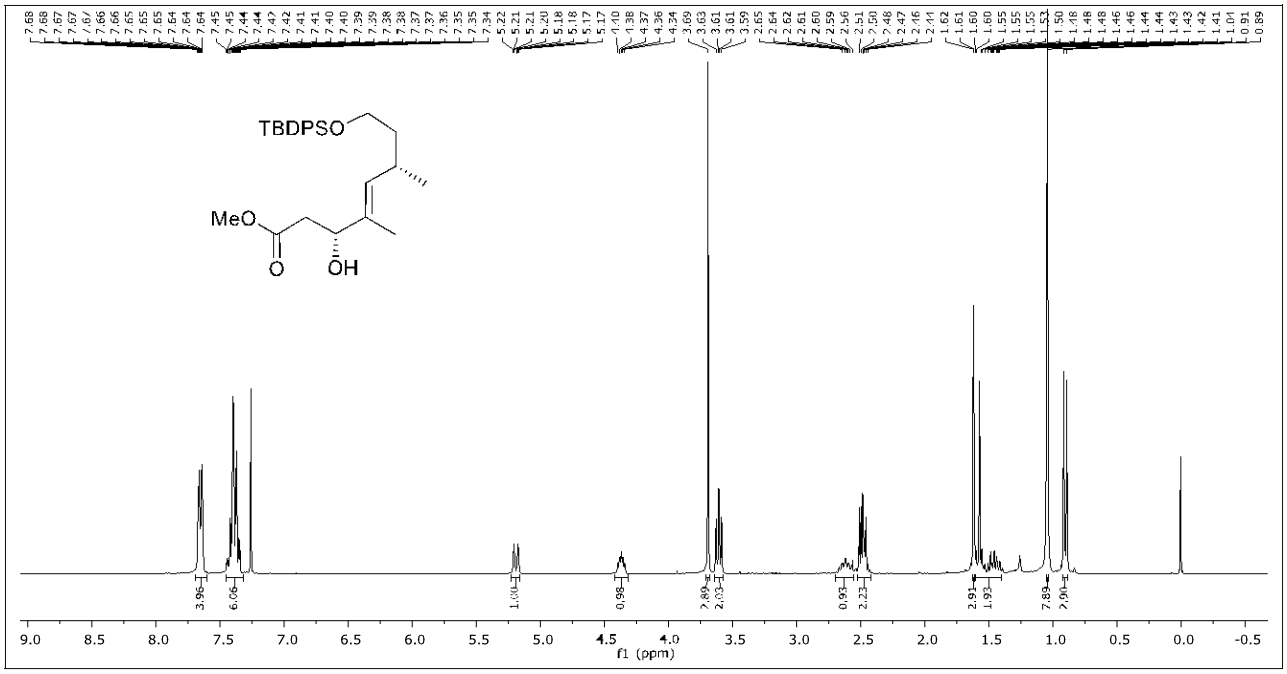
^{1}H-NMR Spectrum of Compound 20a (300 MHz, $CDCl_3$):



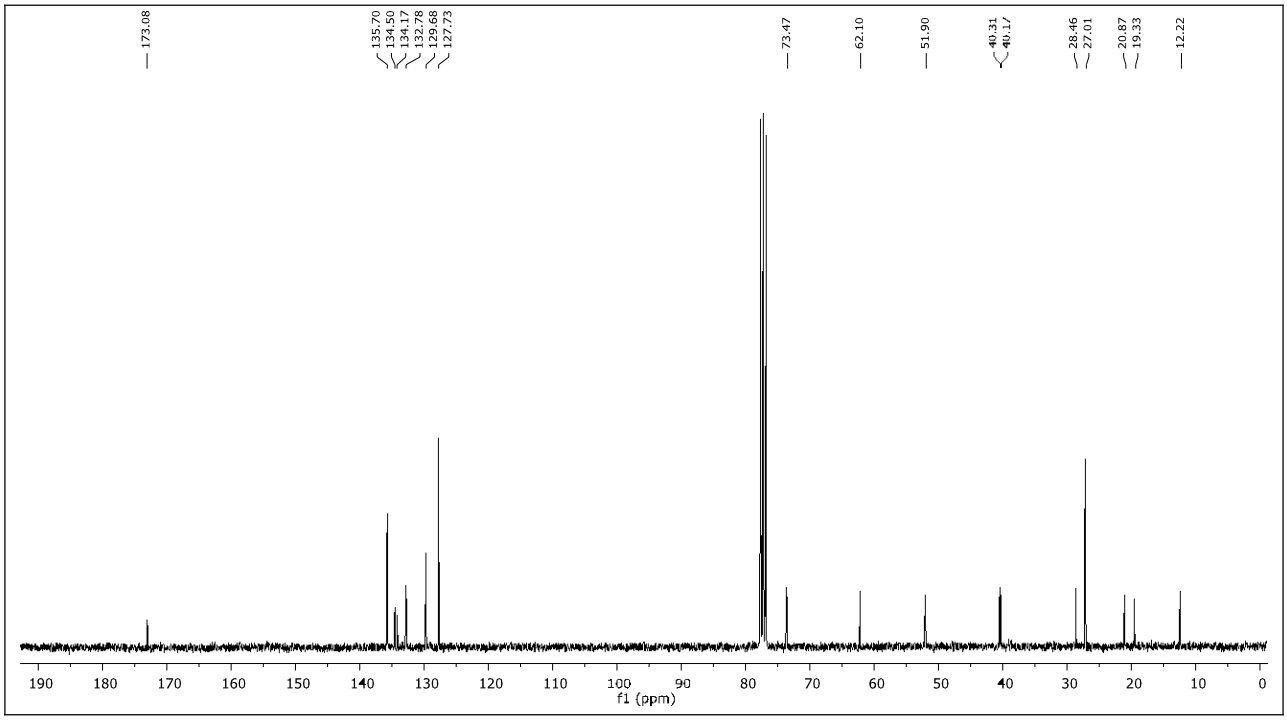
^{13}C-NMR Spectrum of Compound 20a (300 MHz, $CDCl_3$):



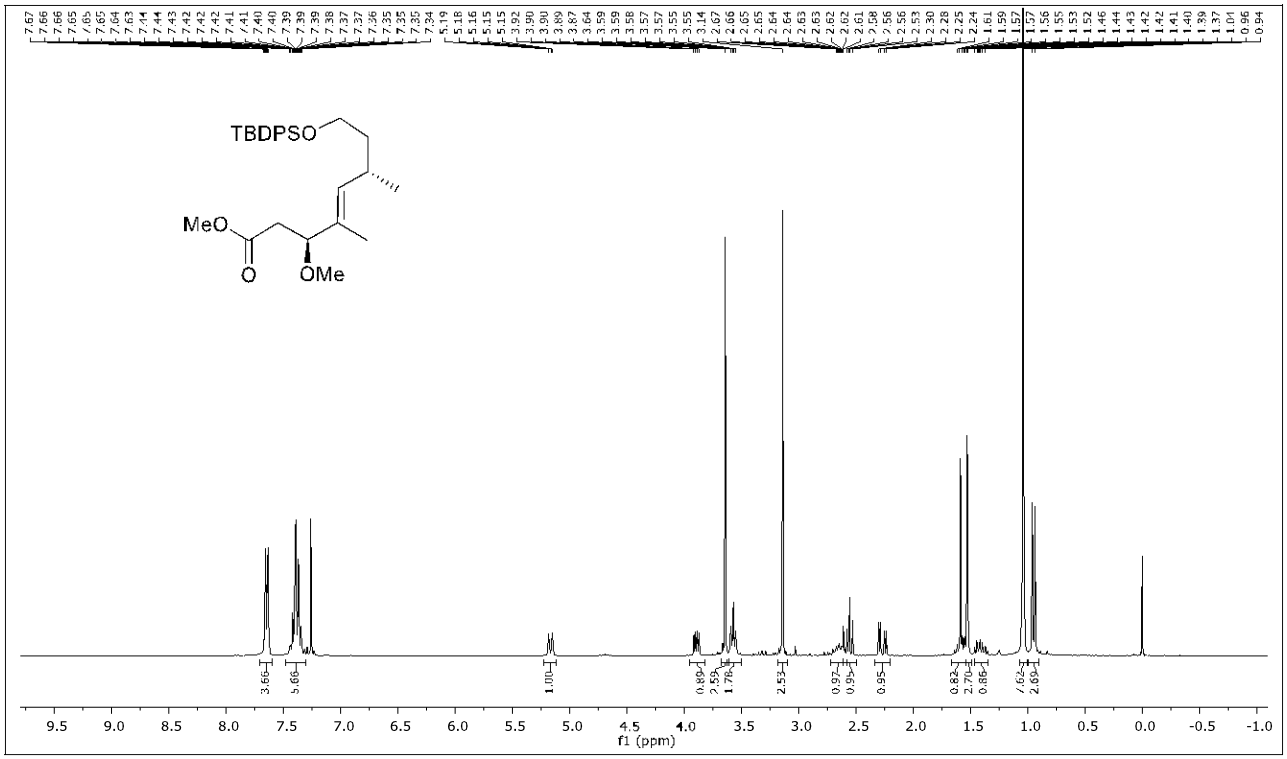
^{1}H-NMR Spectrum of Compound 20b (300 MHz, $CDCl_3$):



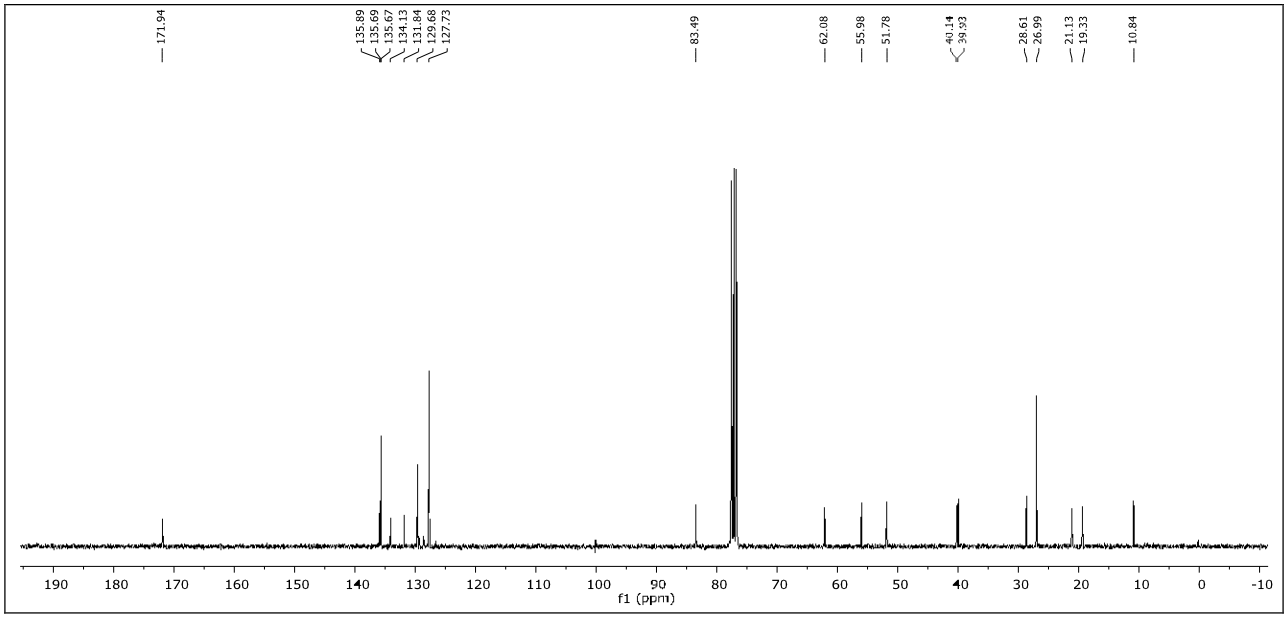
^{13}C-NMR Spectrum of Compound 20b (300 MHz, $CDCl_3$):



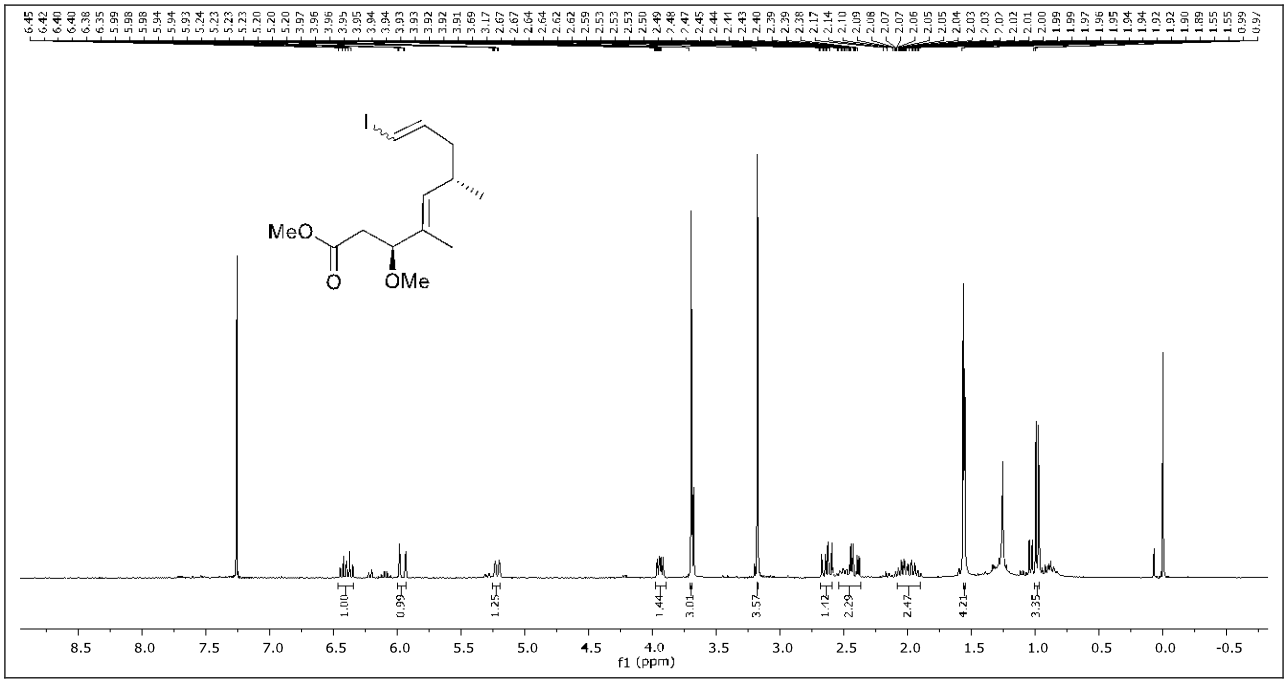
^{1}H-NMR Spectrum of Compound 21 (300 MHz, $CDCl_3$):



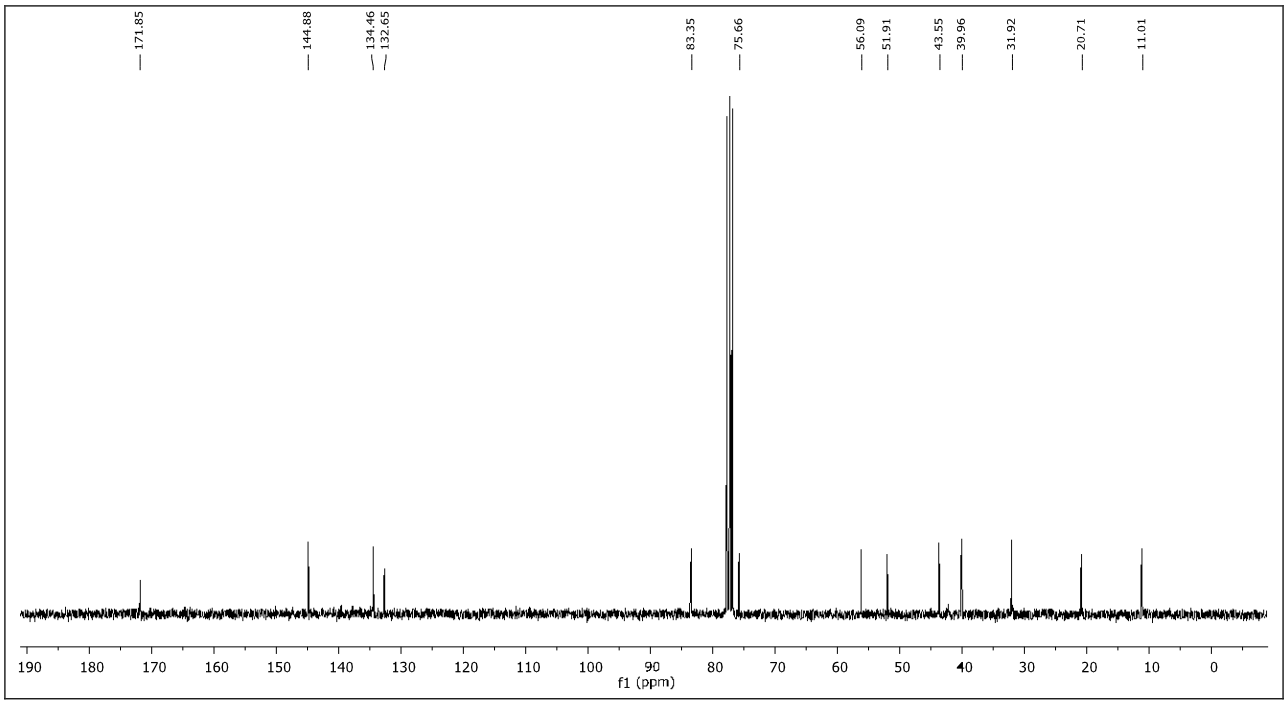
^{13}C-NMR Spectrum of Compound 21 (300 MHz, $CDCl_3$):



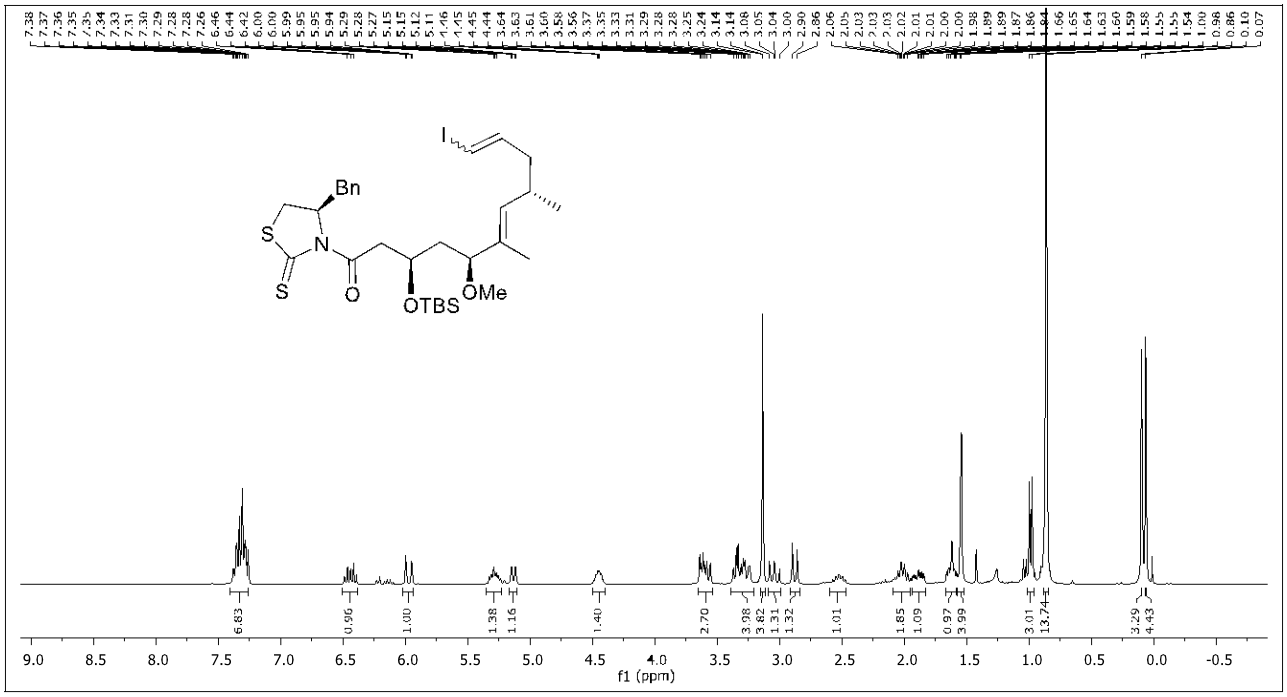
^{1}H-NMR Spectrum of Compound 15 (300 MHz, $CDCl_3$):



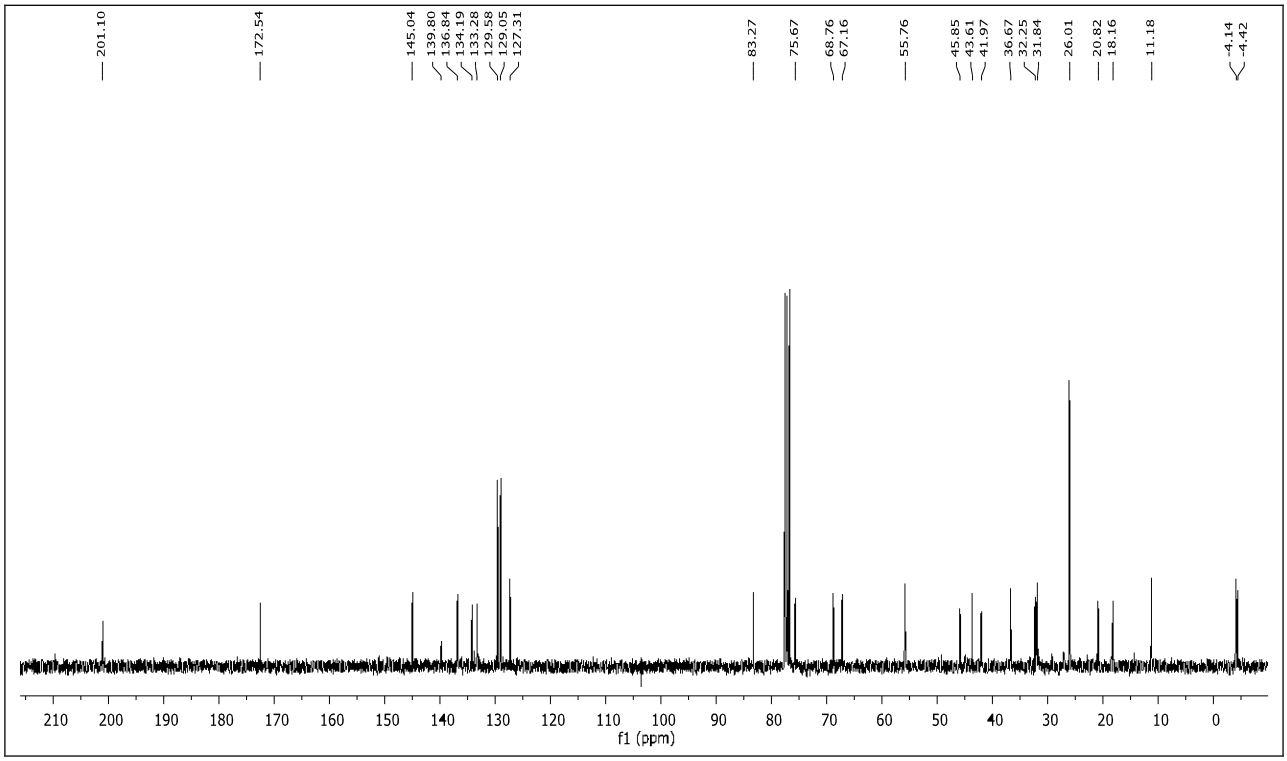
^{13}C-NMR Spectrum of Compound 15 (75 MHz, $CDCl_3$):



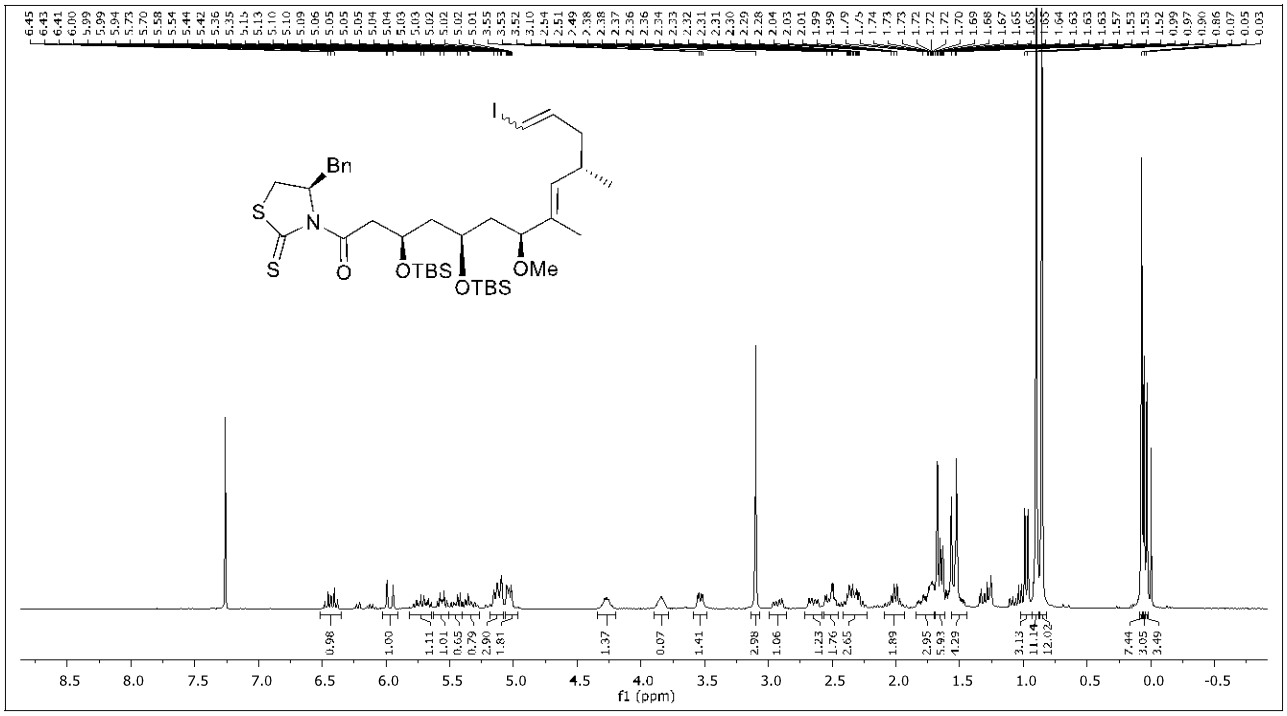
^{1}H-NMR Spectrum of Compound 22 (300 MHz, $CDCl_3$):



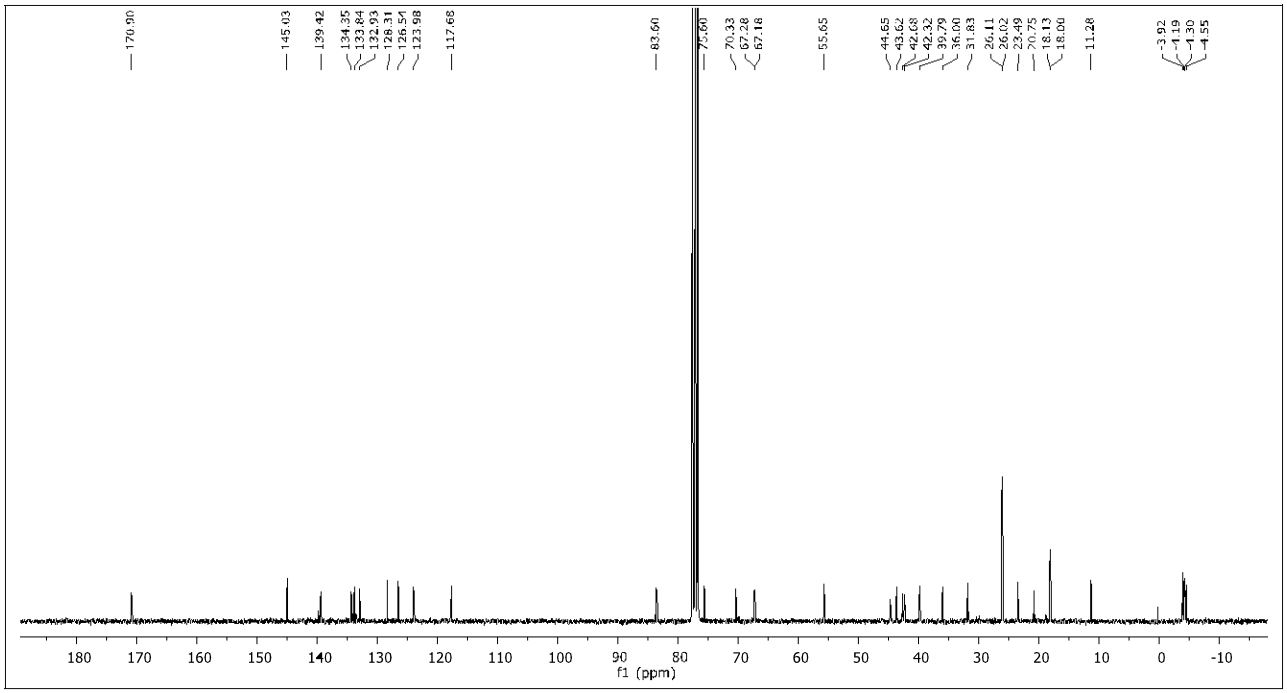
^{13}C-NMR Spectrum of Compound 22 (75 MHz, $CDCl_3$):



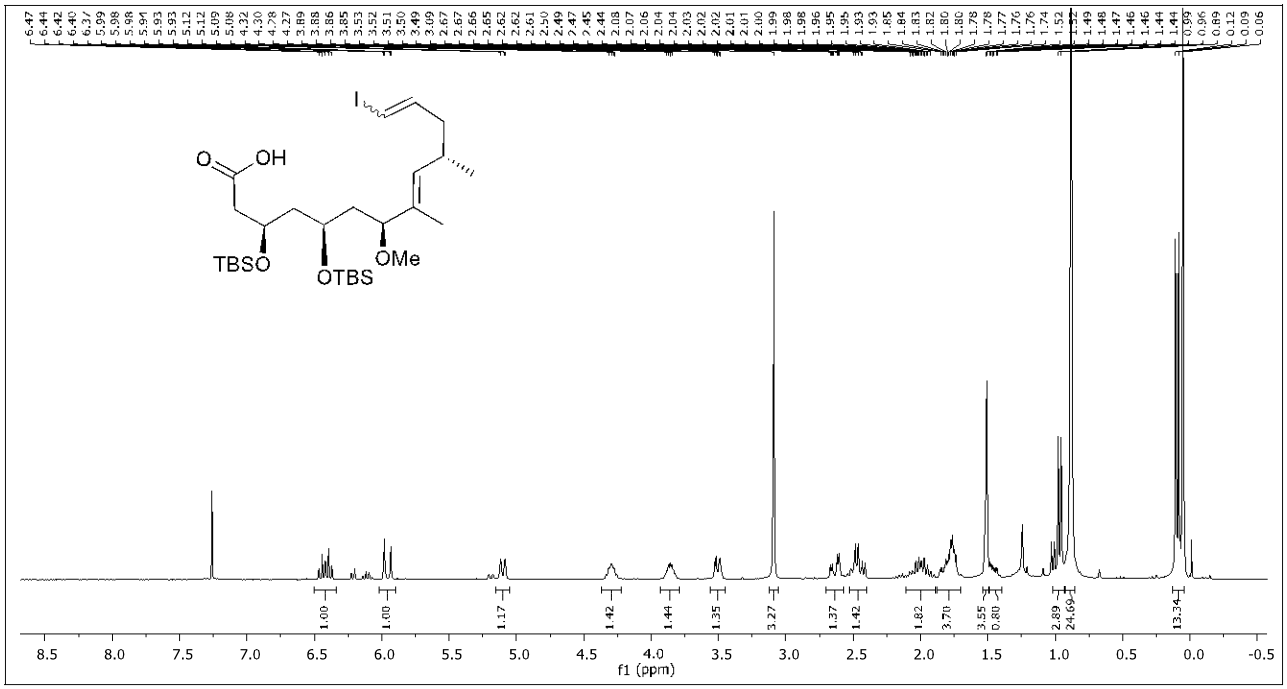
^{1}H-NMR Spectrum of Compound 23 (300 MHz, $CDCl_3$):



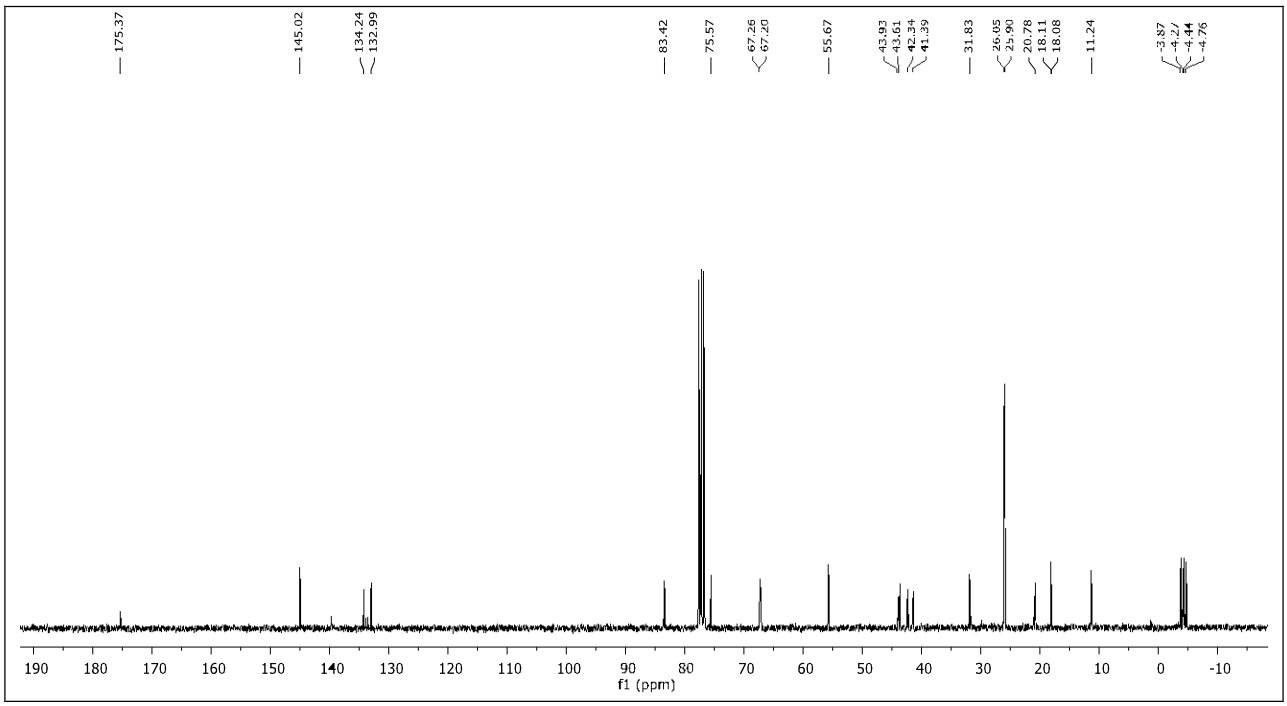
^{13}C-NMR Spectrum of Compound 23 (75 MHz, $CDCl_3$):



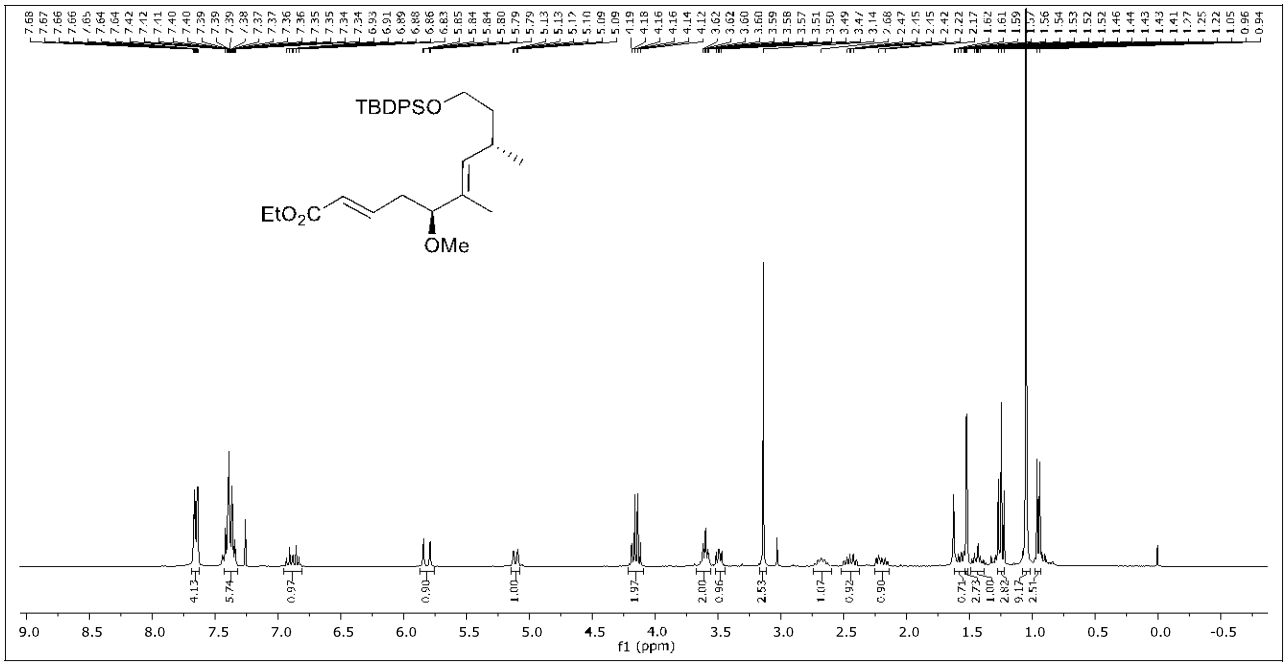
^{1}H-NMR Spectrum of Compound 13 (300 MHz, $CDCl_3$):



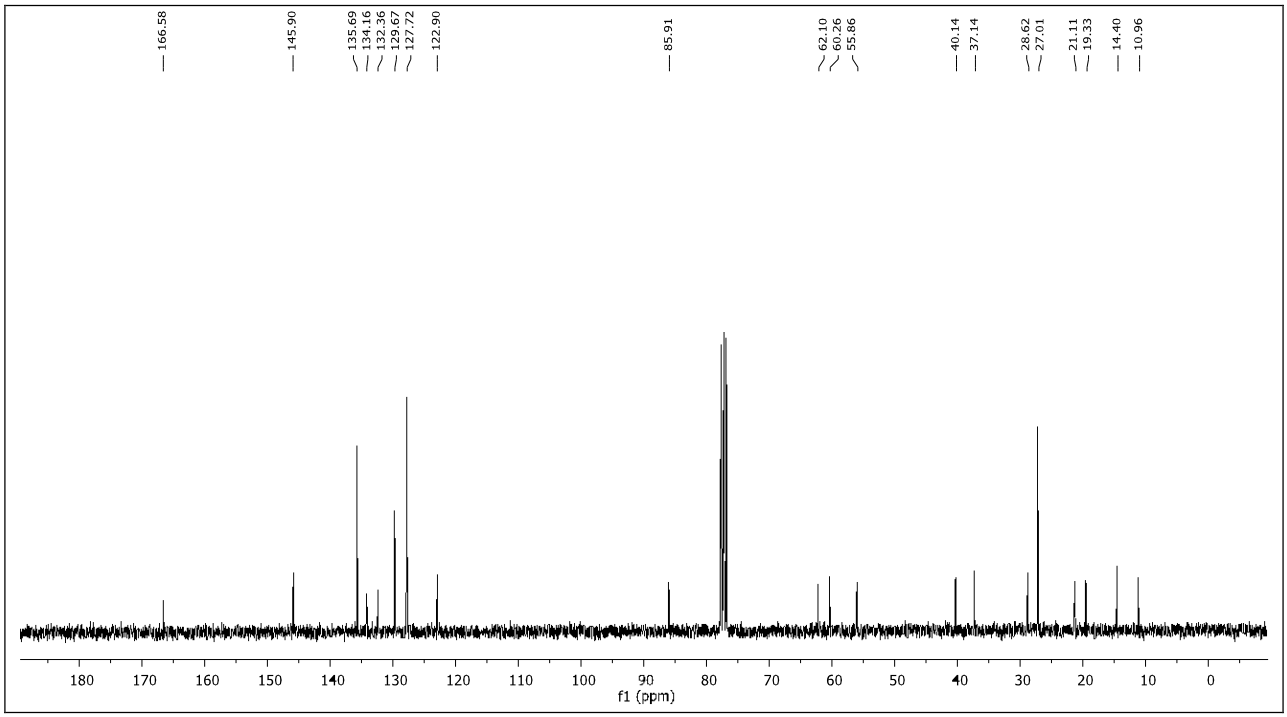
^{13}C-NMR Spectrum of Compound 13 (75 MHz, $CDCl_3$):



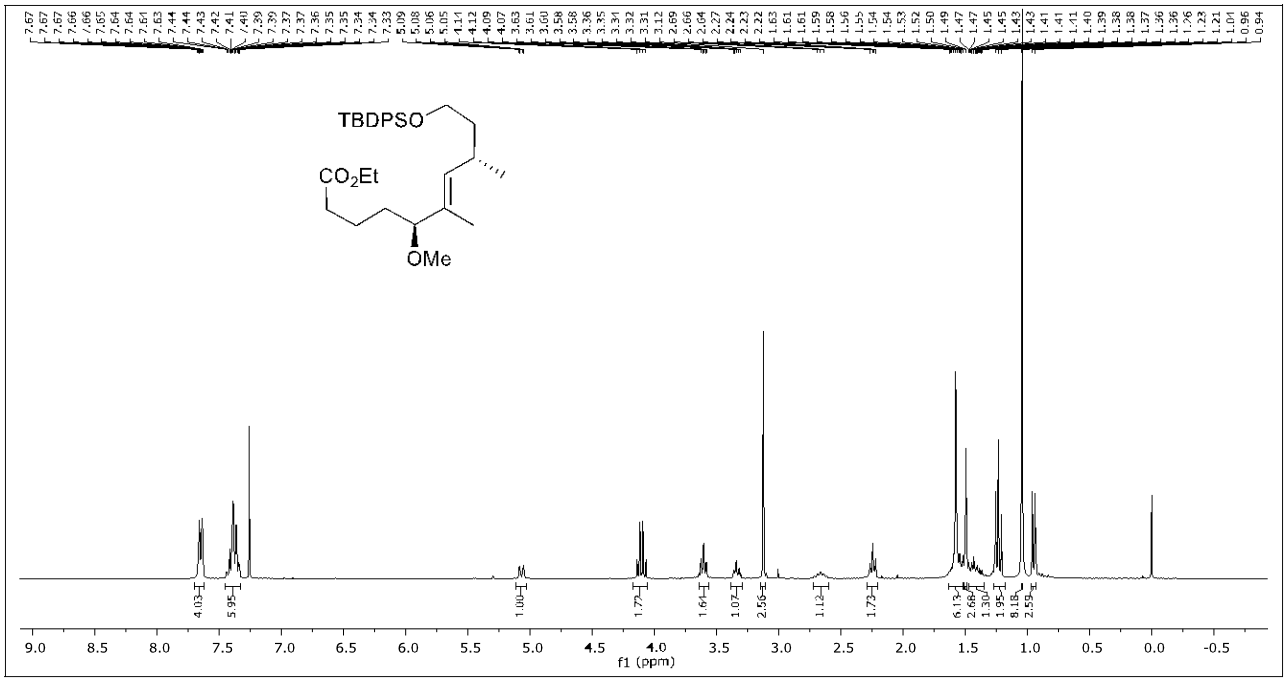
^{1}H-NMR Spectrum of Compound 24 (300 MHz, $CDCl_3$):



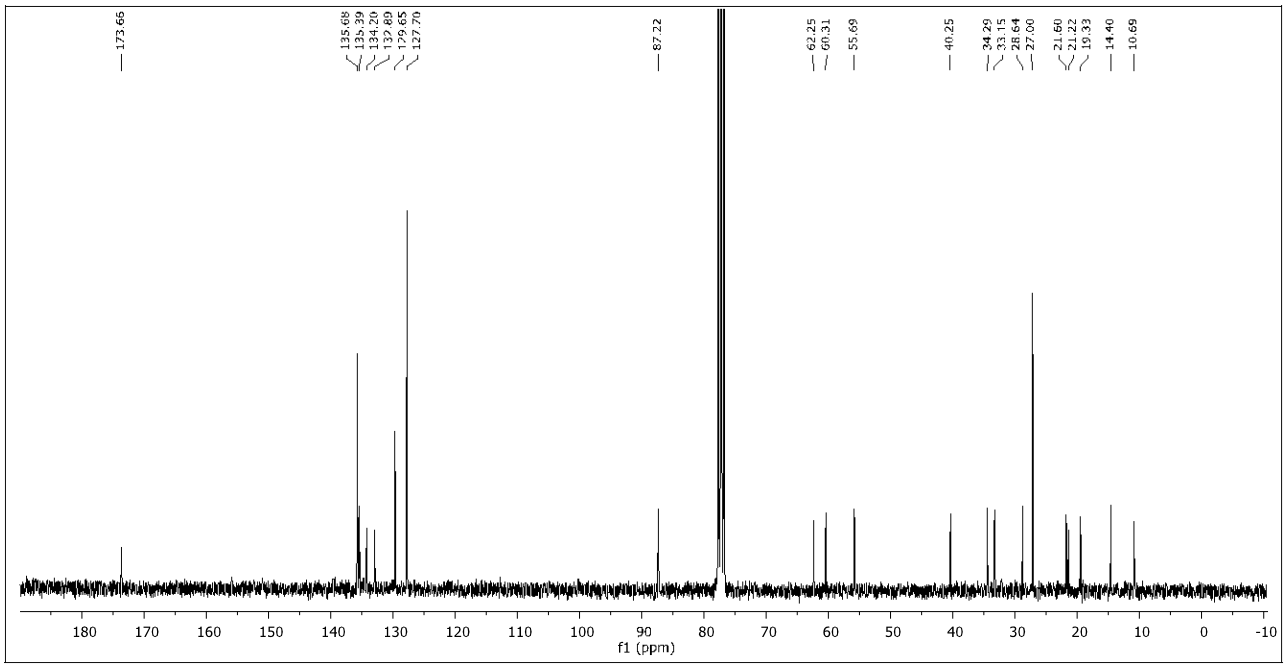
^{13}C-NMR Spectrum of Compound 24 (75 MHz, $CDCl_3$):



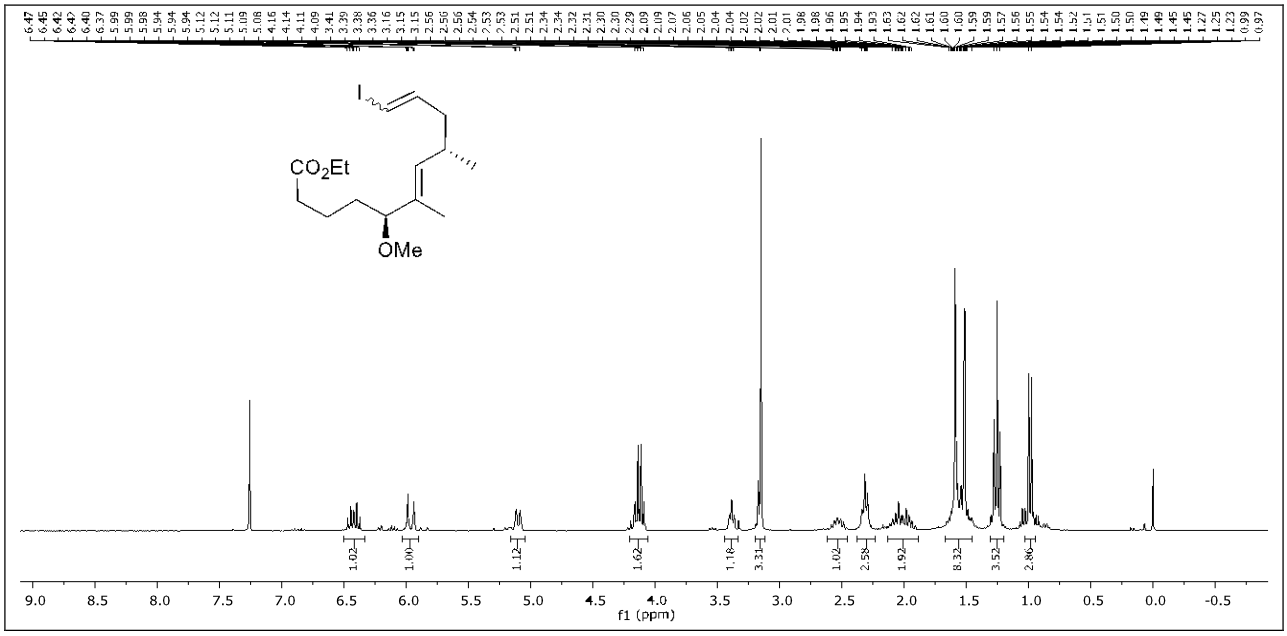
^{1}H-NMR Spectrum of Compound 25 (300 MHz, $CDCl_3$):



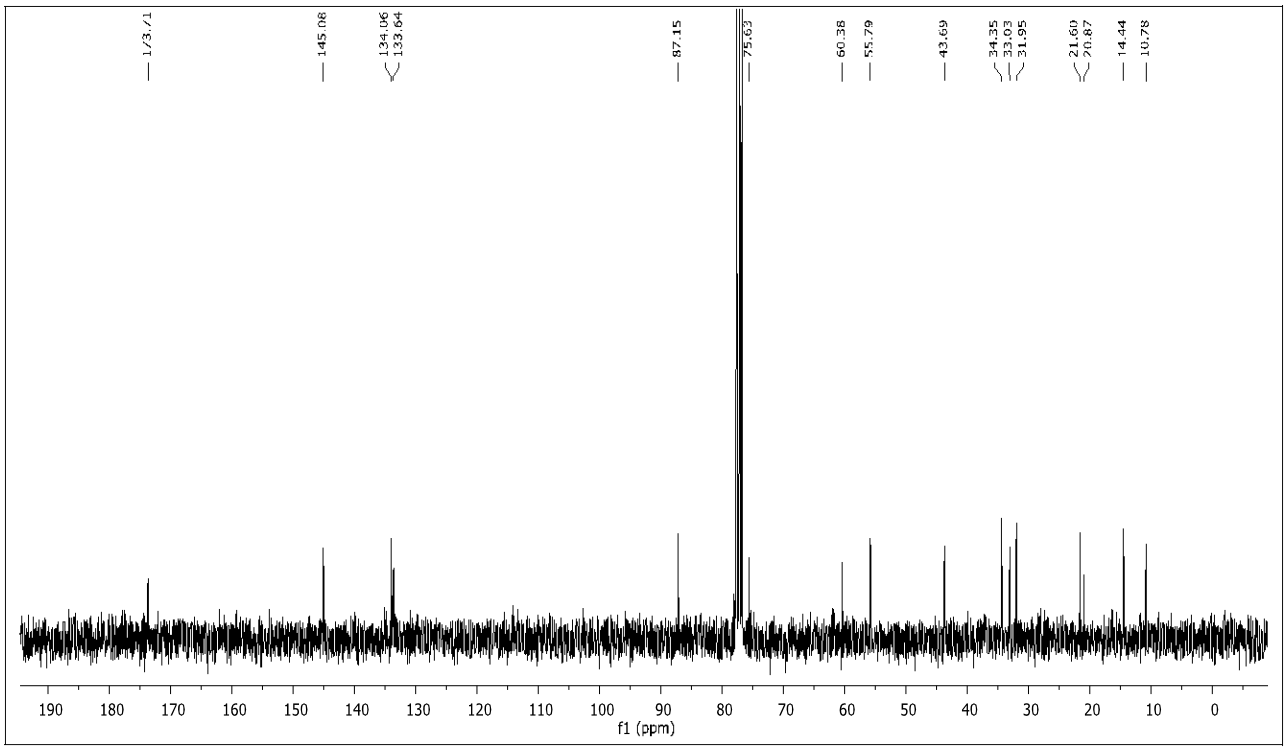
^{13}C-NMR Spectrum of Compound 25 (75 MHz, $CDCl_3$):



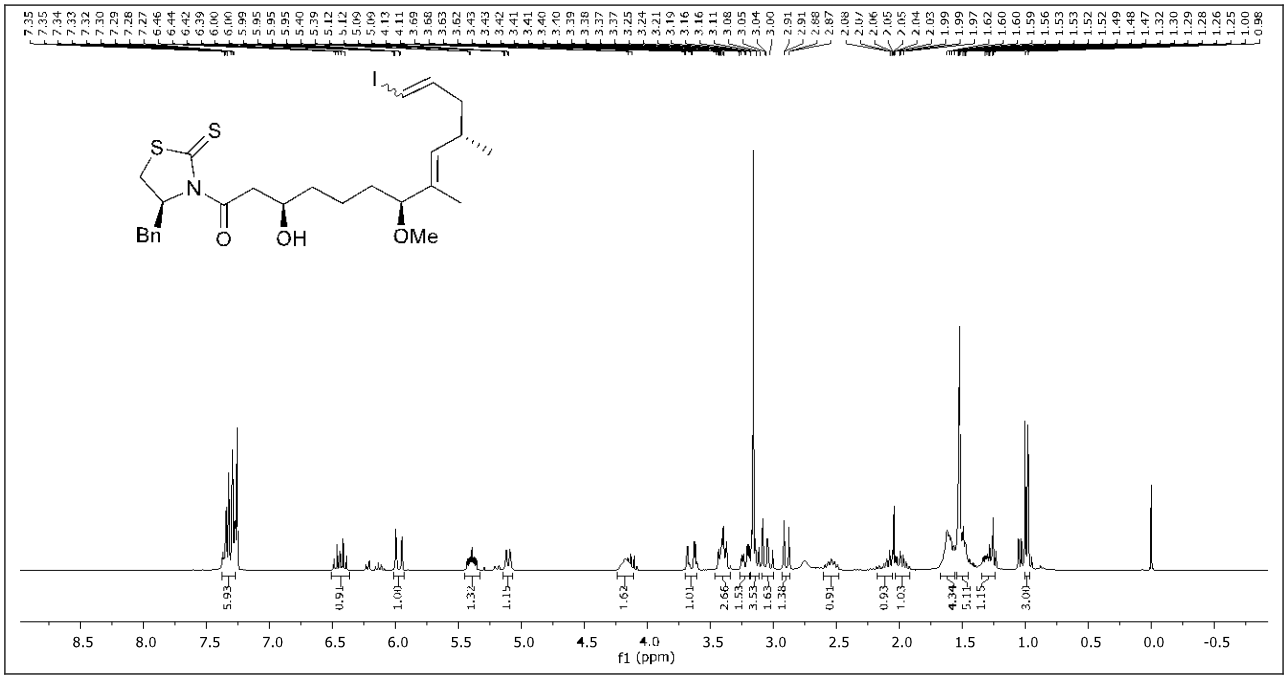
^{1}H-NMR Spectrum of Compound 26 (300 MHz, $CDCl_3$):



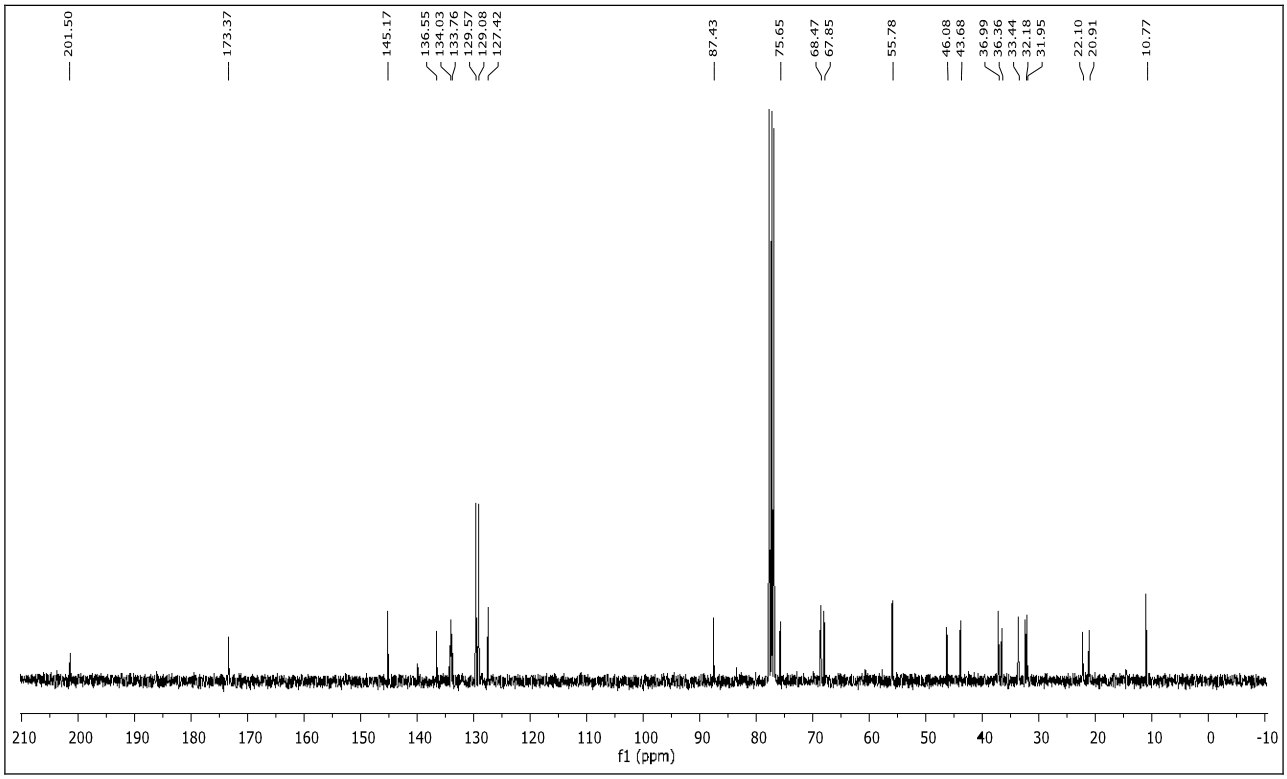
^{13}C-NMR Spectrum of Compound 26 (75 MHz, $CDCl_3$):



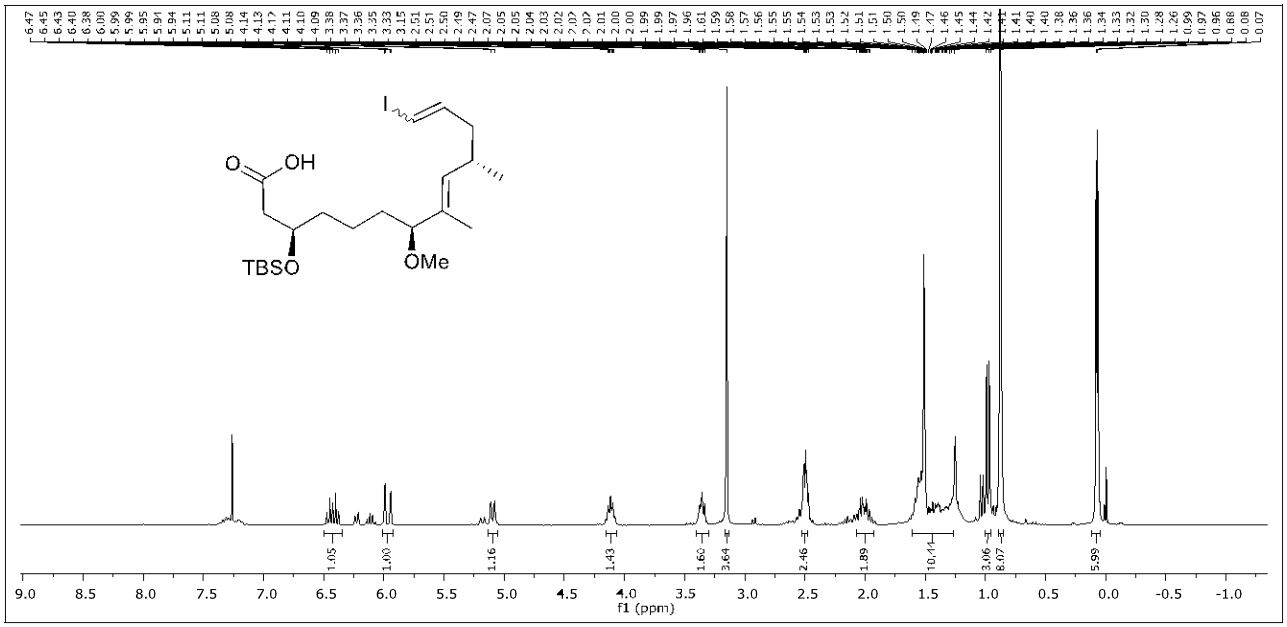
^{1}H-NMR Spectrum of Compound 27 (300 MHz, $CDCl_3$):



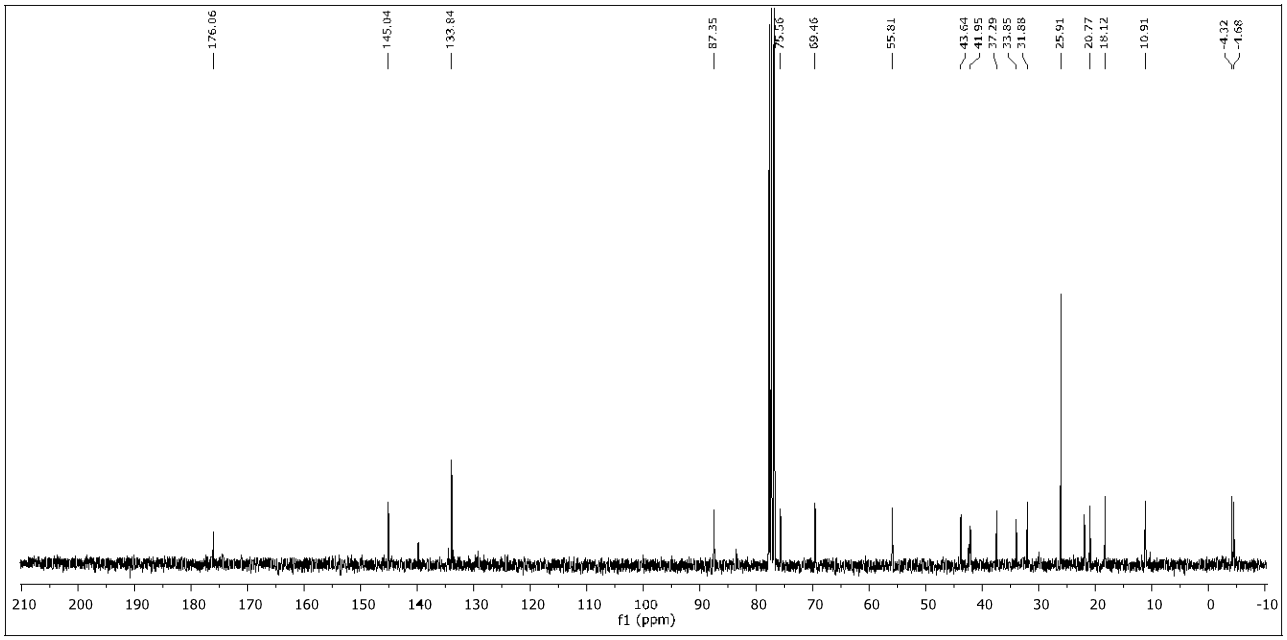
^{13}C-NMR Spectrum of Compound 27 (75 MHz, $CDCl_3$):



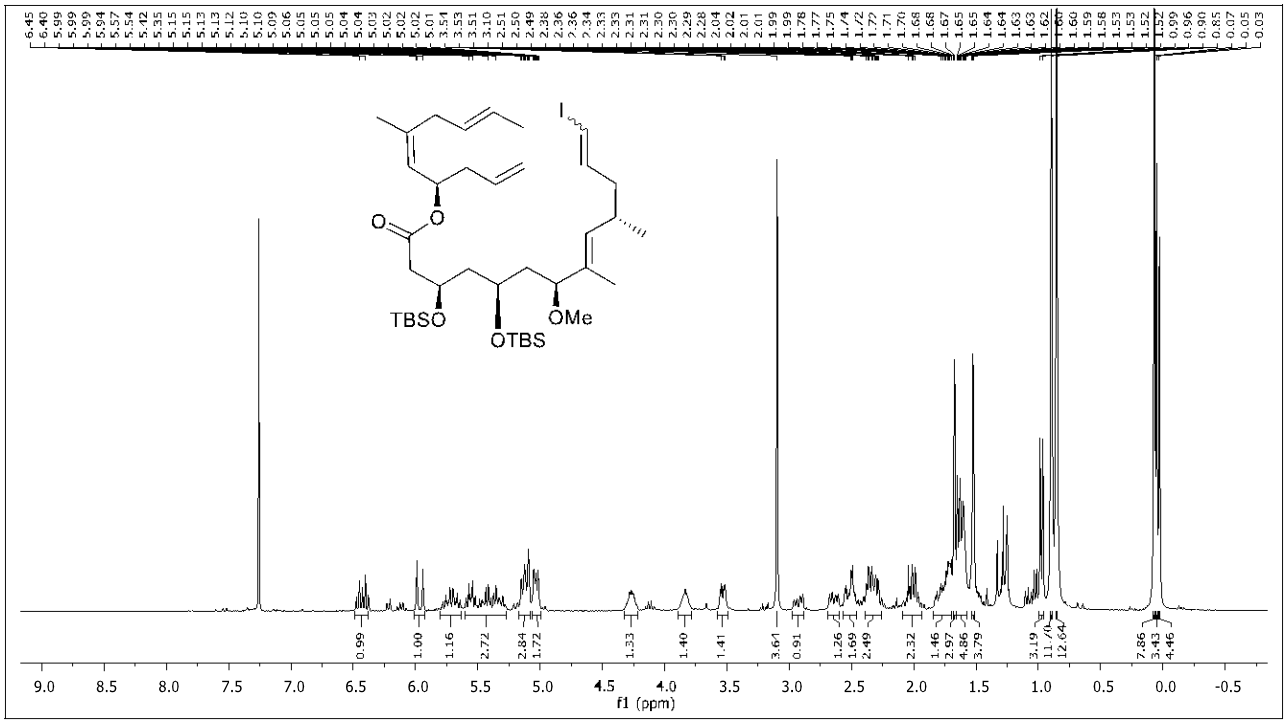
^{1}H-NMR Spectrum of Compound 14 (300 MHz, $CDCl_3$):



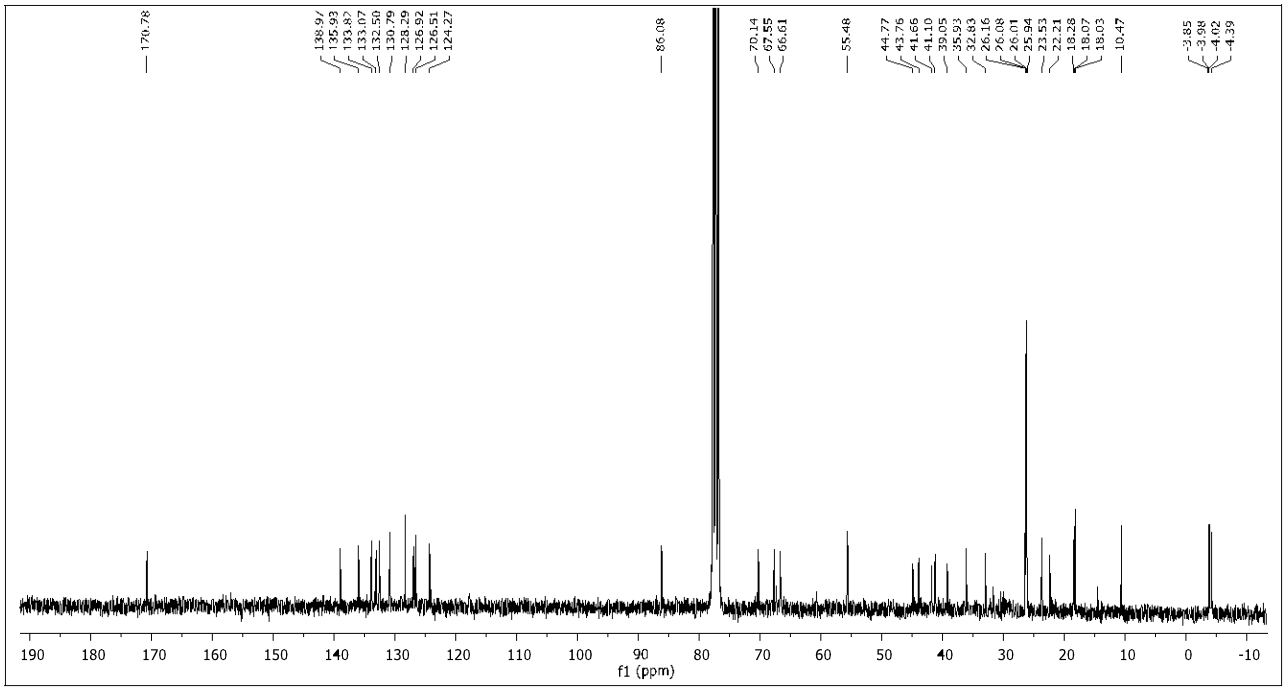
^{13}C-NMR Spectrum of Compound 14 (75 MHz, $CDCl_3$):



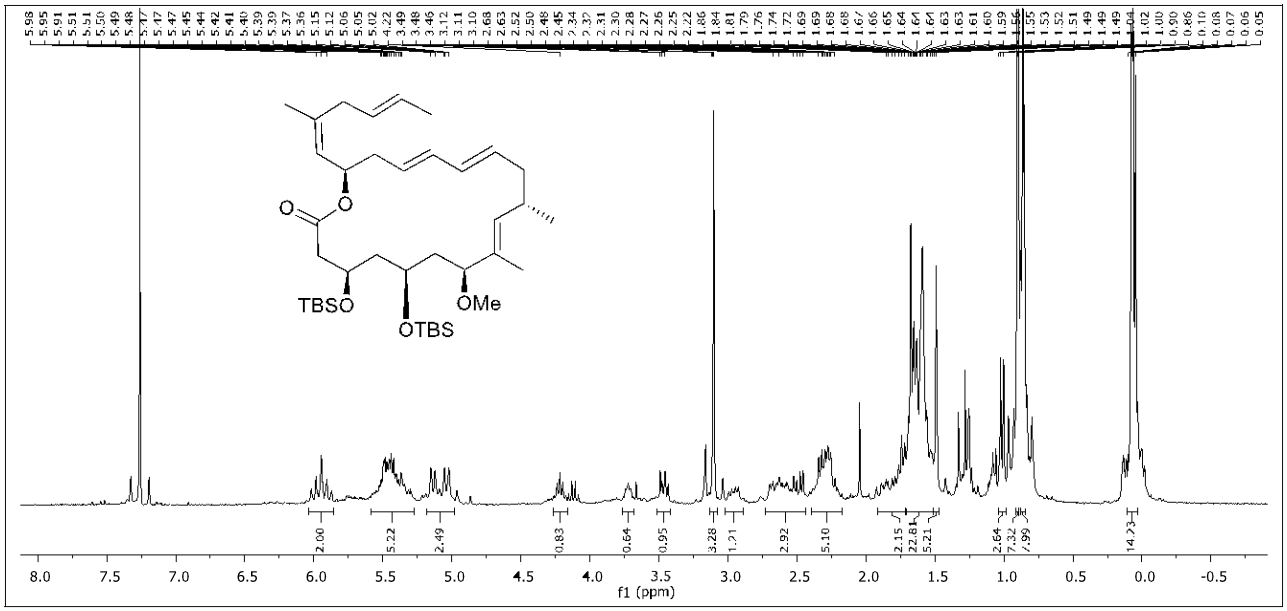
^{1}H-NMR Spectrum of Compound 10 (300 MHz, $CDCl_3$):



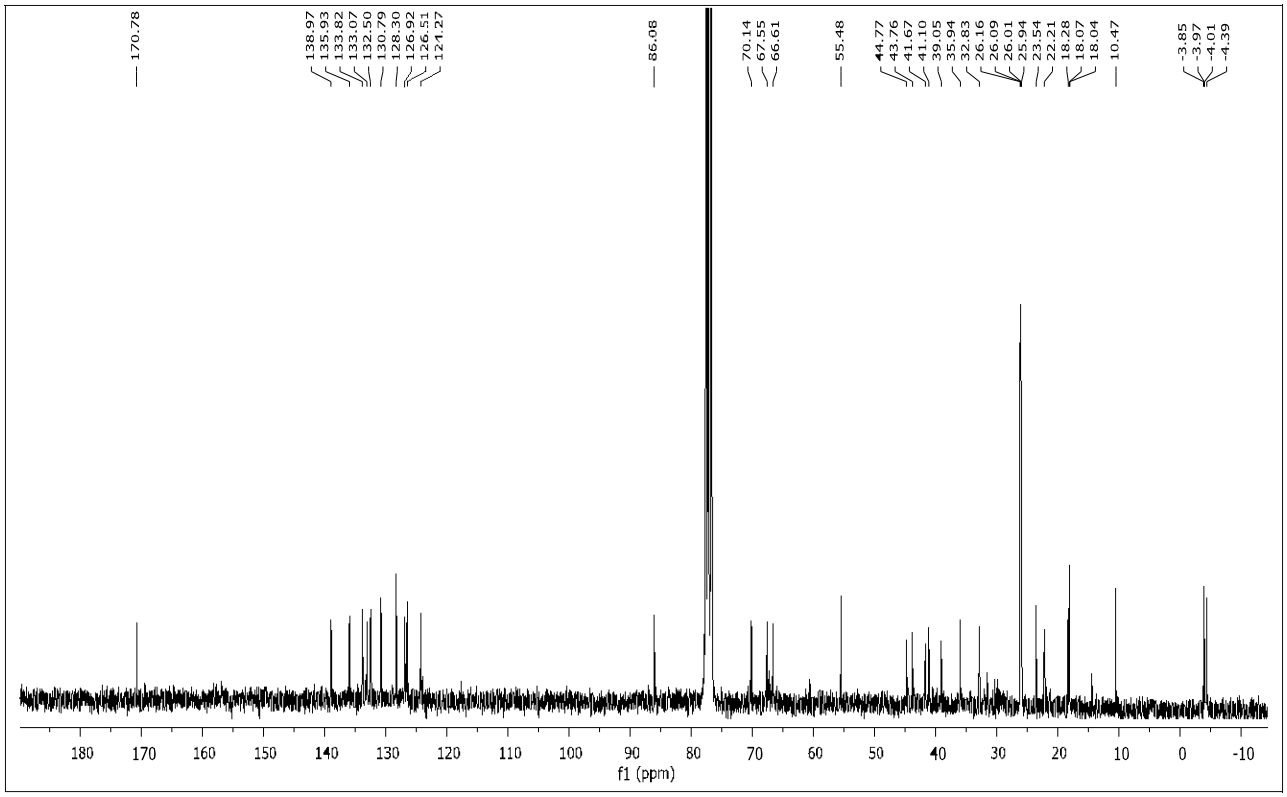
^{13}C-NMR Spectrum of Compound 10 (75 MHz, $CDCl_3$):



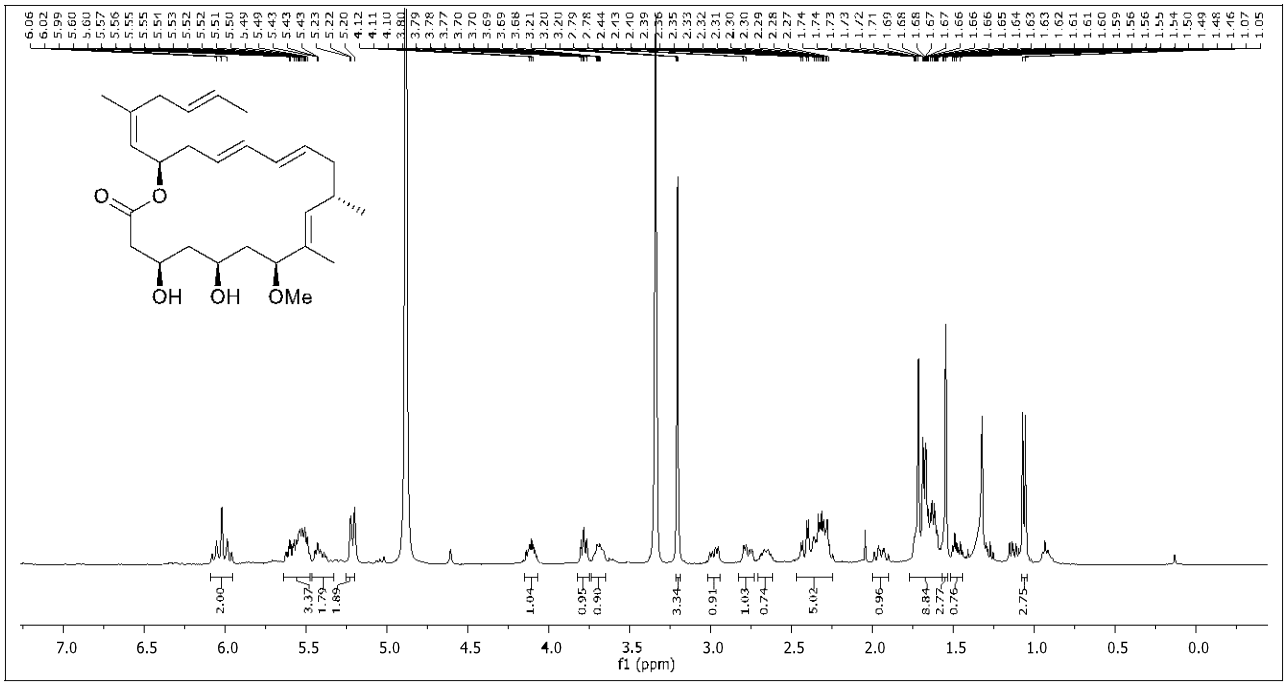
^{1}H-NMR Spectrum of Compound 29 (300 MHz, $CDCl_3$):



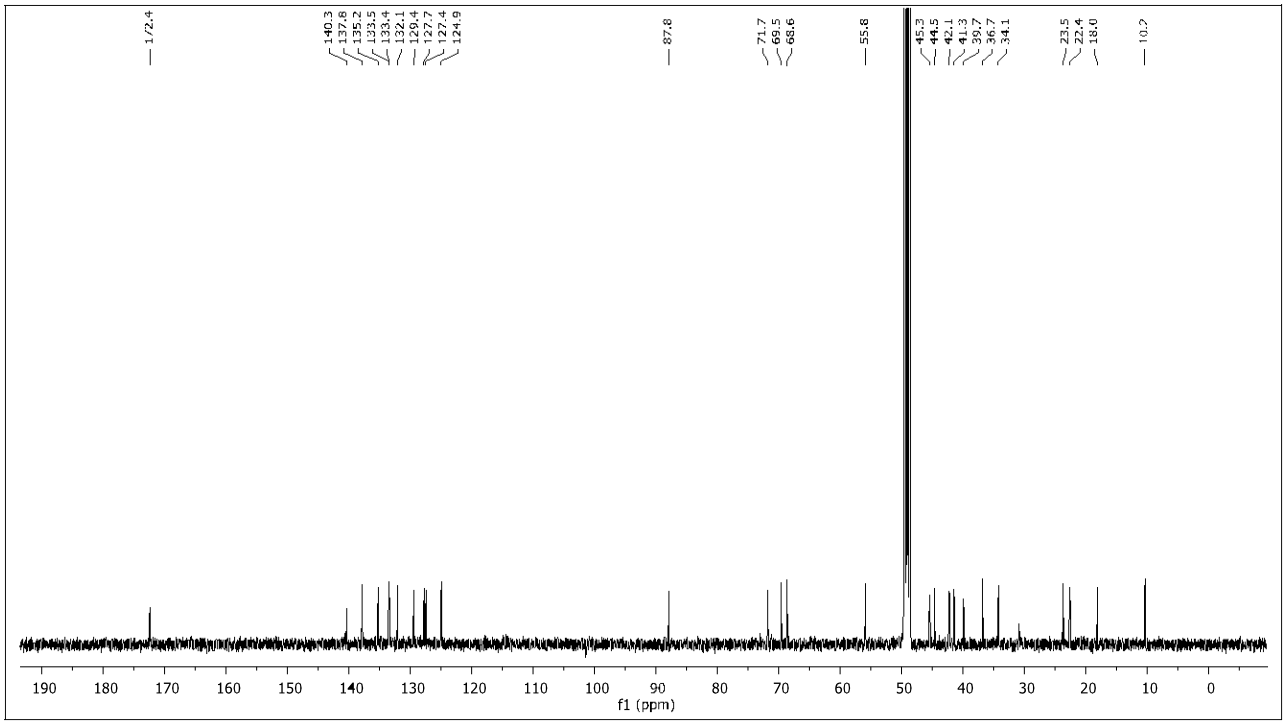
^{13}C-NMR Spectrum of Compound 29 (75 MHz, $CDCl_3$):



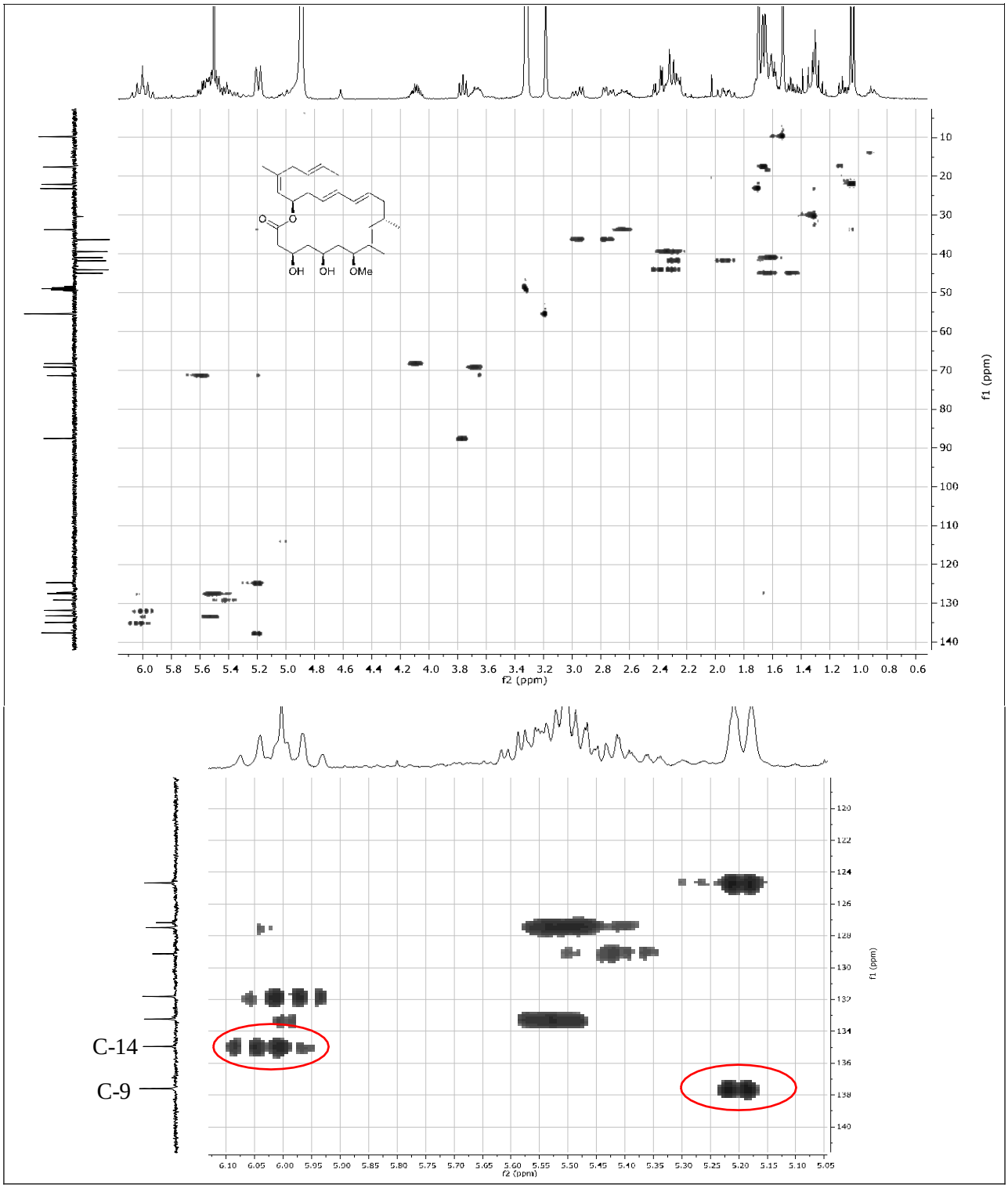
^{1}H-NMR Spectrum of Biselyngbyolide A (4) (500 MHz, CD_3OD):



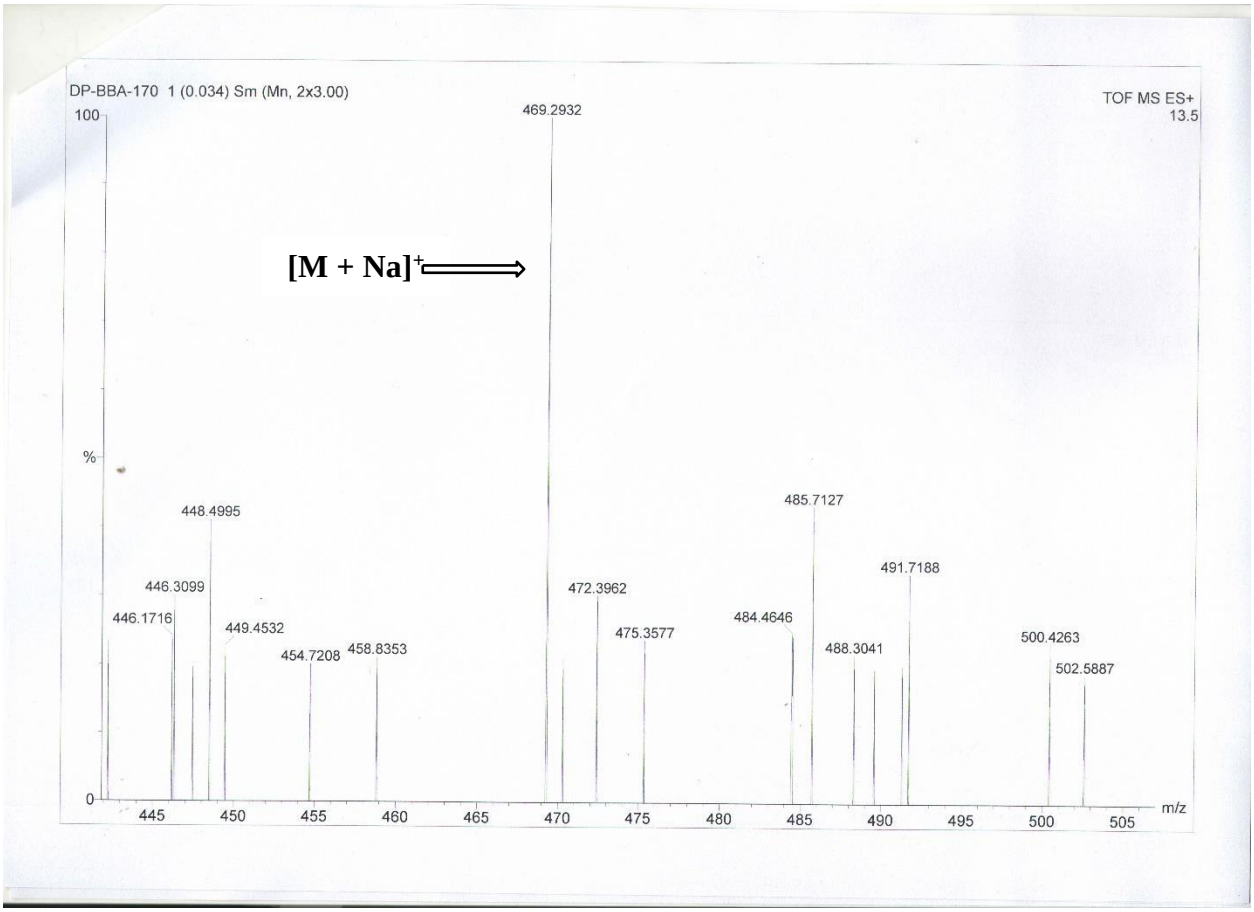
^{13}C-NMR Spectrum of Biselyngbyolide A (4) (125 MHz, CD_3OD):



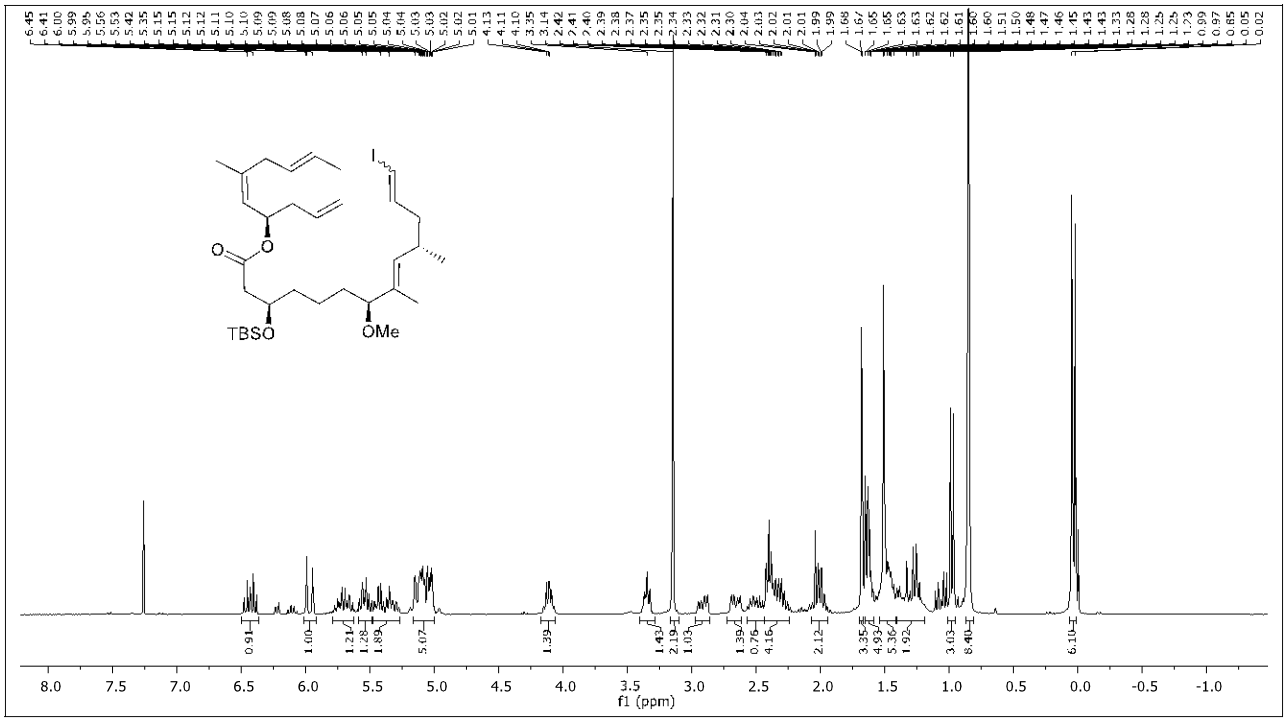
HSQC Spectrum of Biselyngbyolide A (4) (75 MHz, CD_3OD):



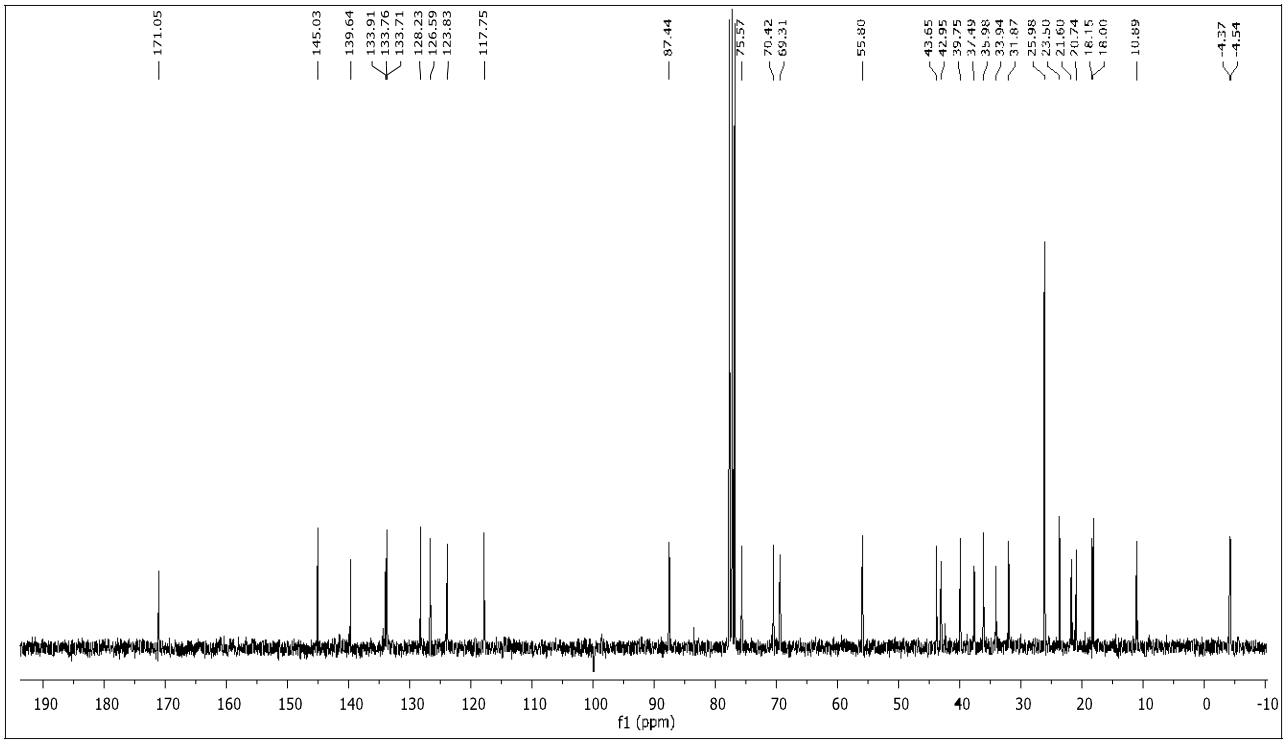
HRMS Spectrum of Biselyngbyolide A (4).



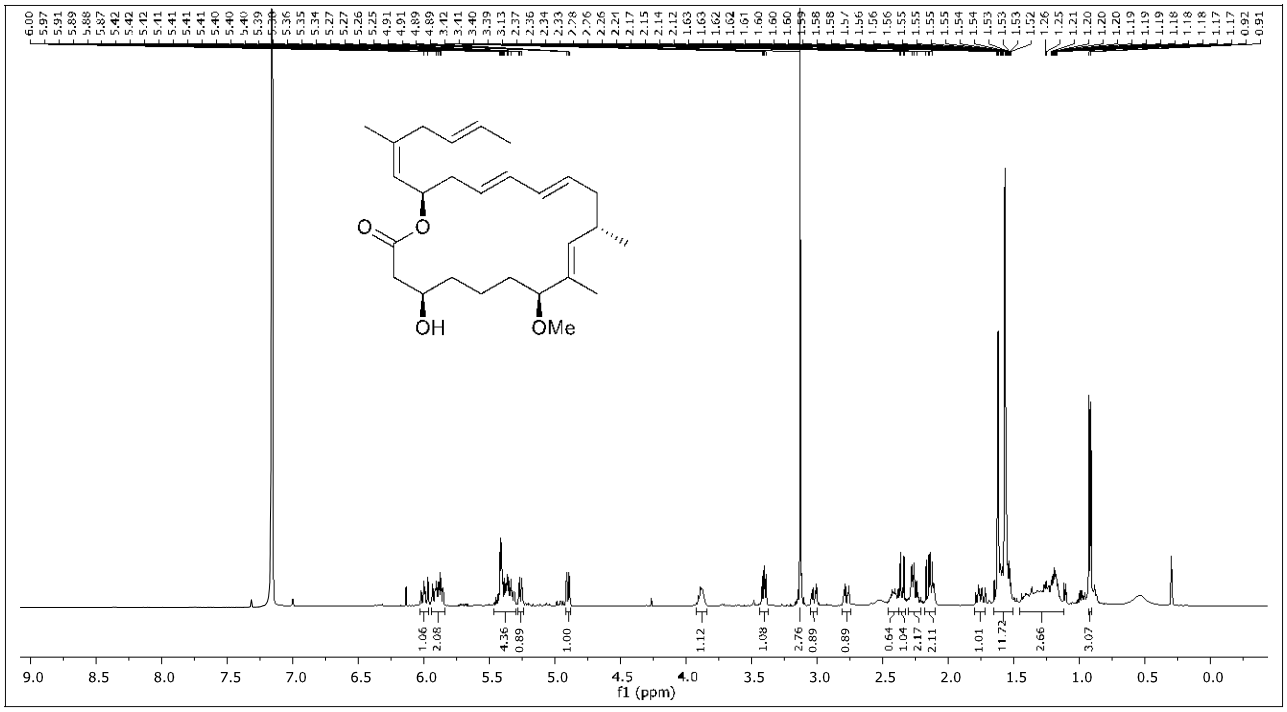
^{1}H-NMR Spectrum of Compound 10 (300 MHz, $CDCl_3$):



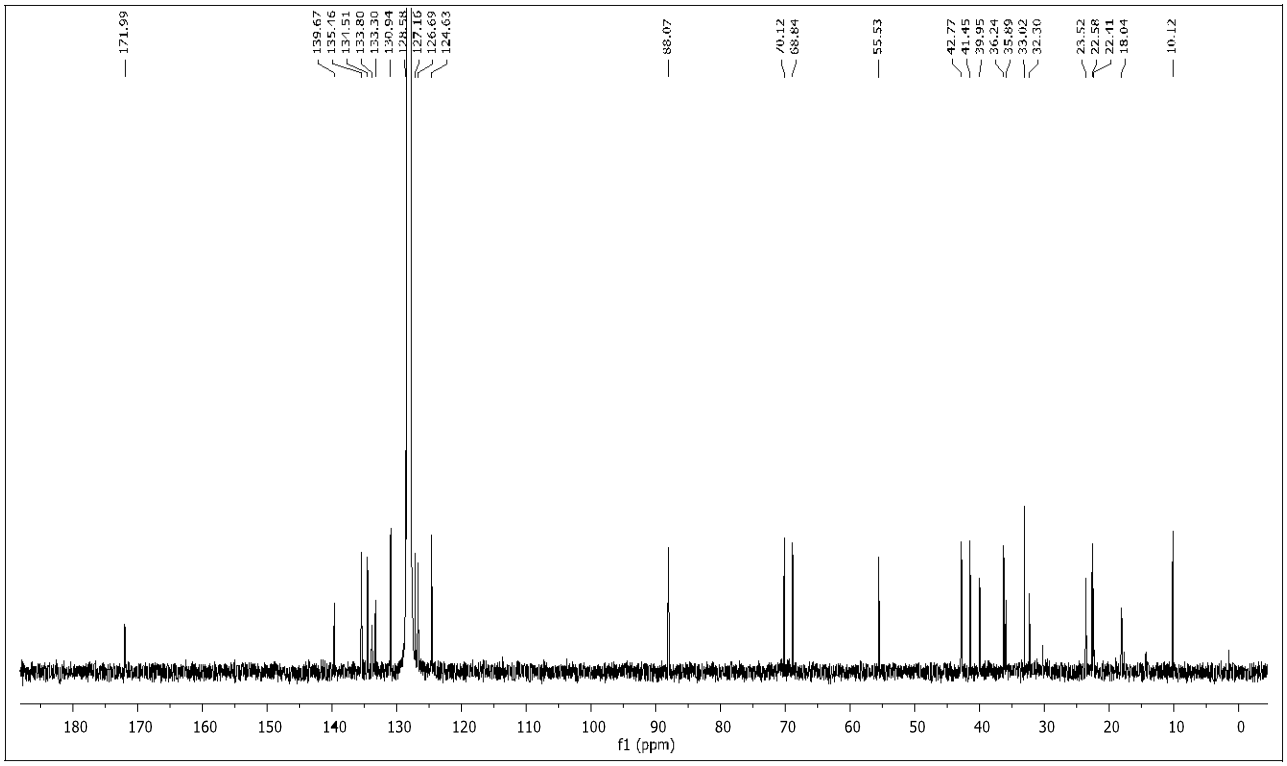
^{13}C-NMR Spectrum of Compound 10 (75 MHz, $CDCl_3$):



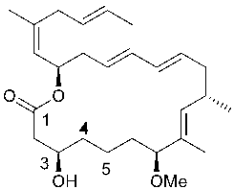
^{1}H-NMR Spectrum of Biselyngbyolide C (6) (500 MHz, C_6D_6):

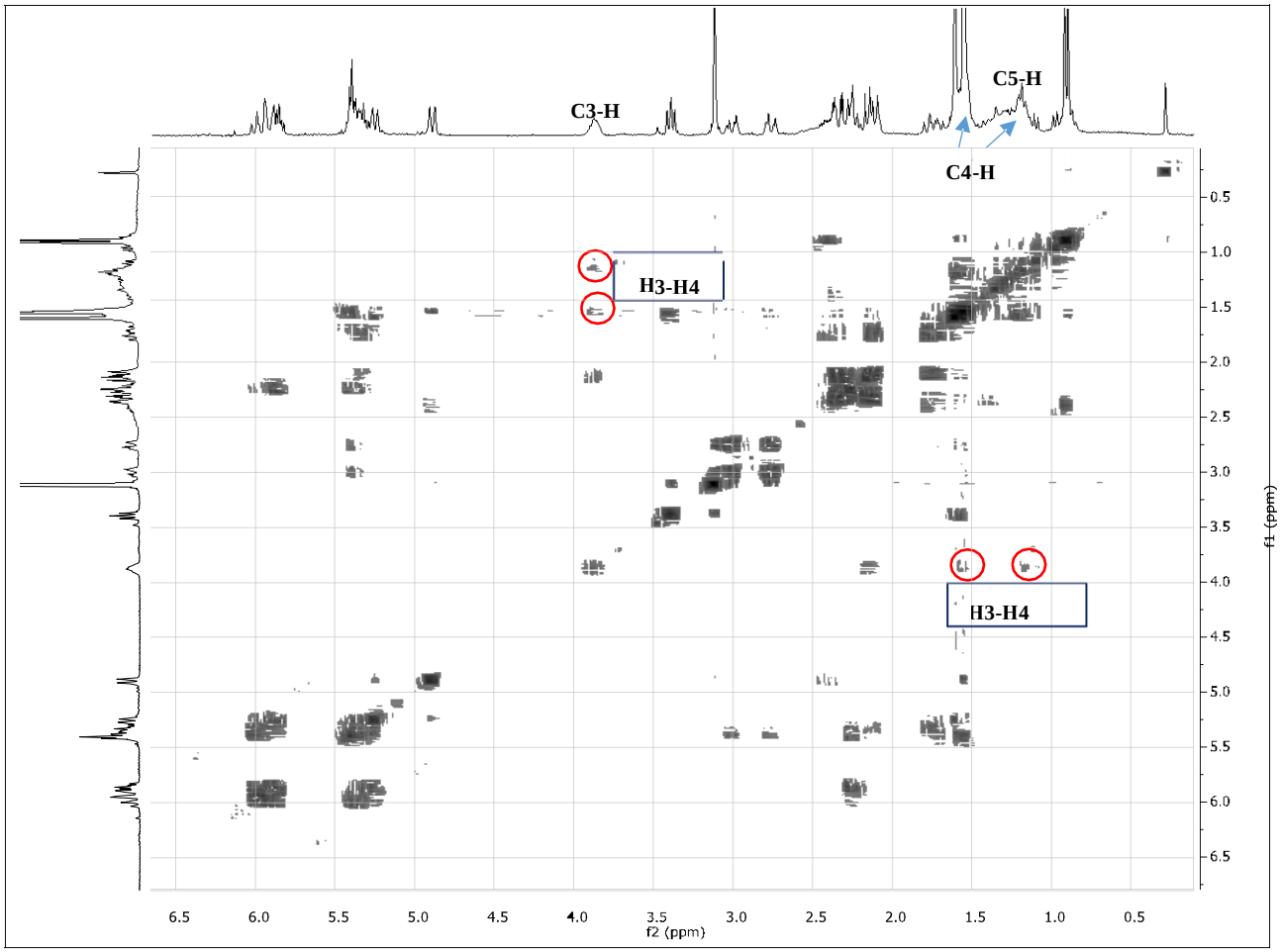


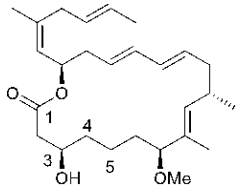
^{13}C-NMR Spectrum of Biselyngbyolide C (6) (125 MHz, C_6D_6):



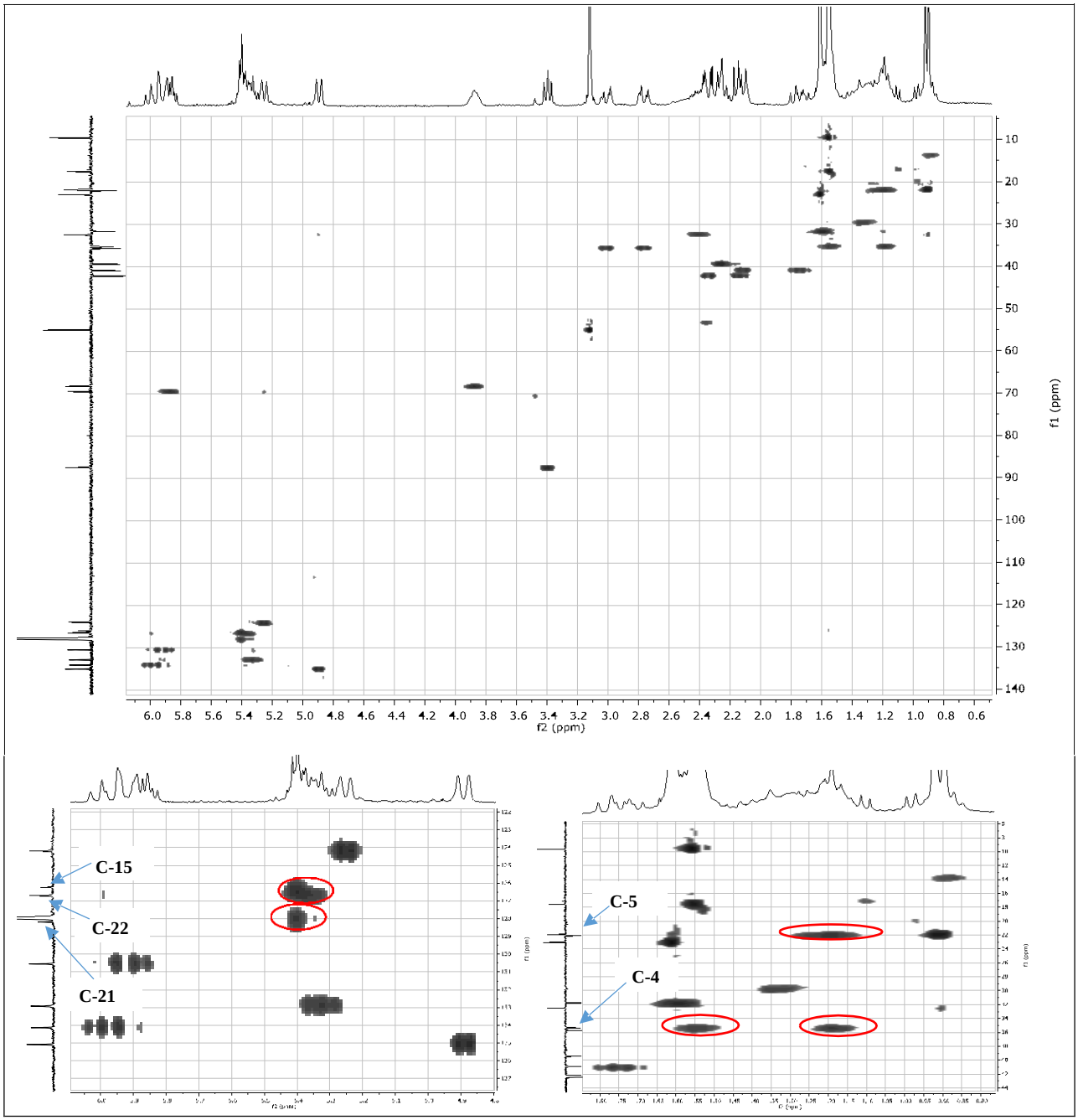
COSY Spectrum of Biselyngbyolide C (6) (300 MHz, C_6D_6):



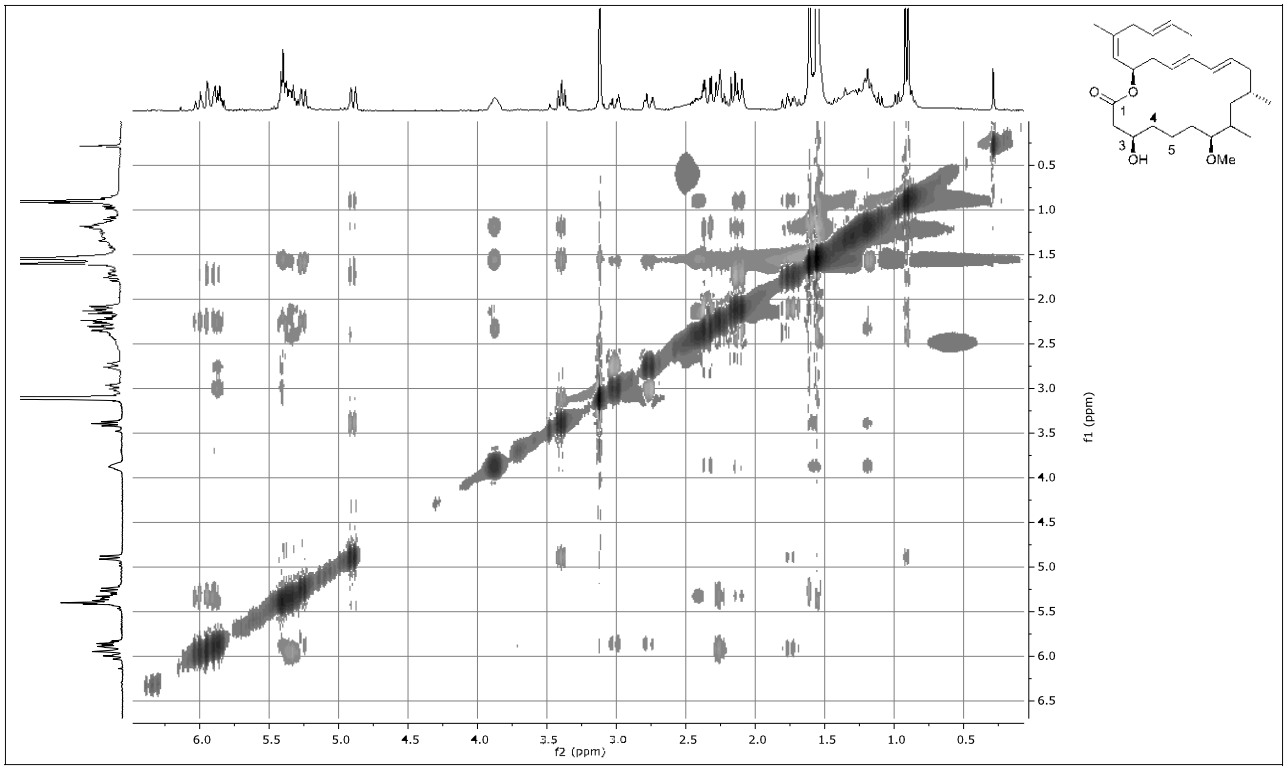




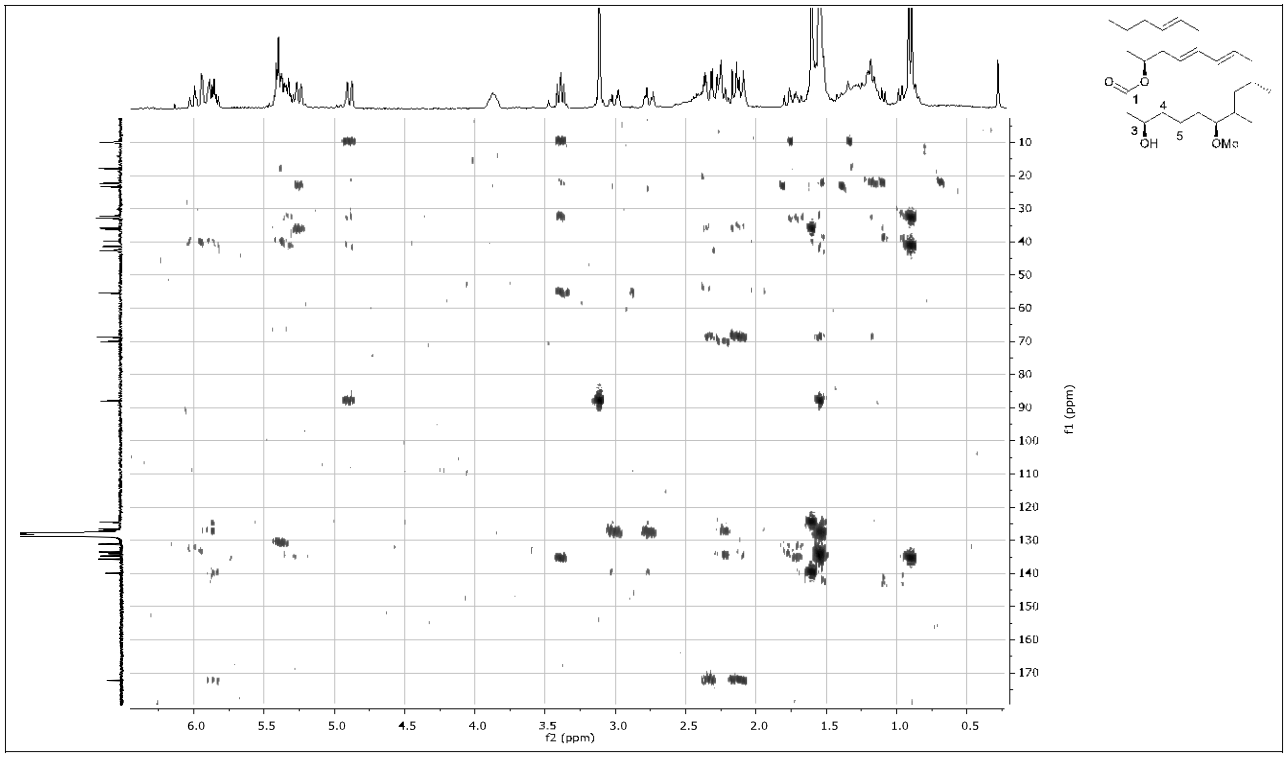
HSQC Spectrum of Biselyngbyolide C (6) (75 MHz, C_6D_6):



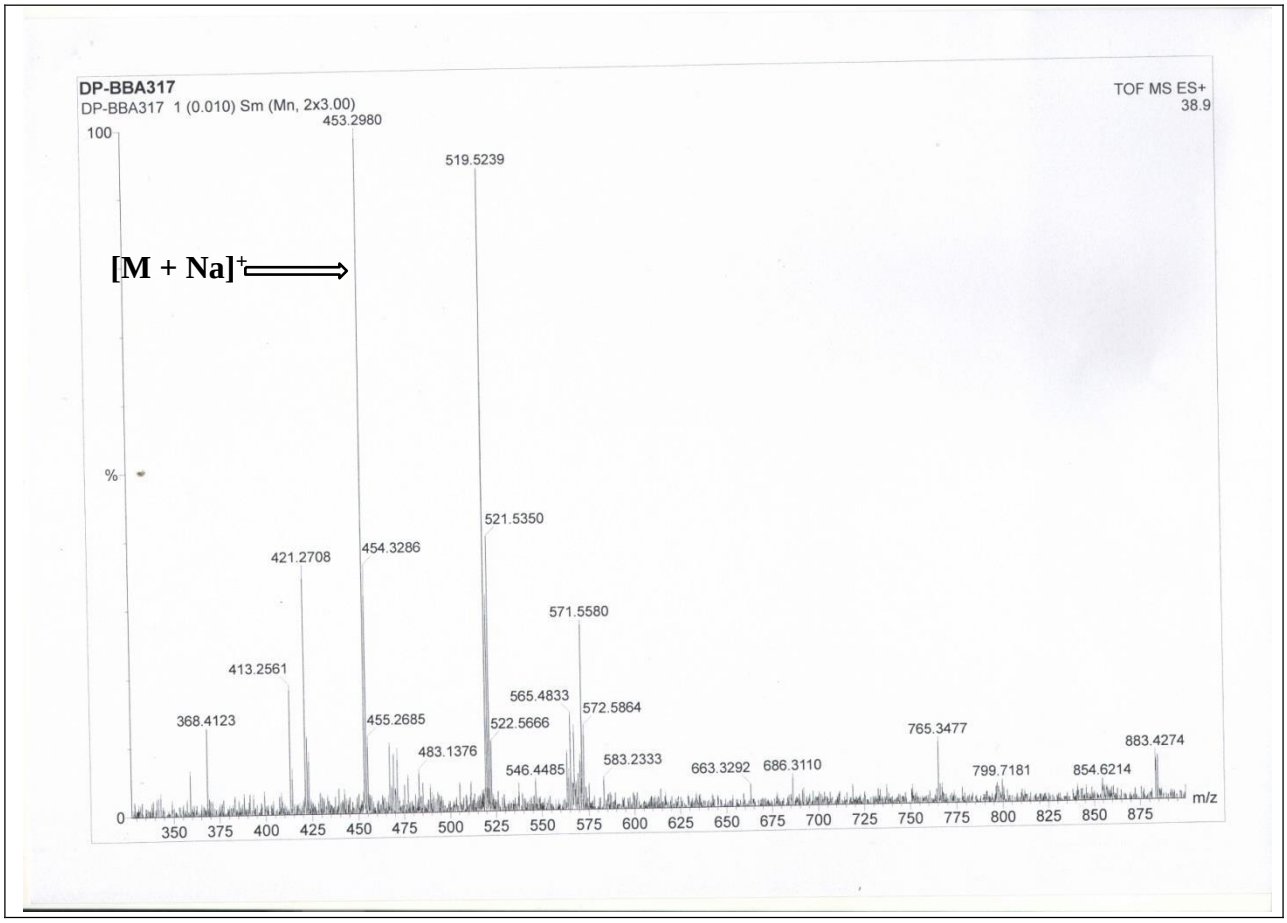
NOESY Spectrum of Biselyngbyolide C (6) (300 MHz, C_6D_6):



HMBC Spectrum of Biselyngbyolide C (6) (300 MHz, C_6D_6):



HRMS spectrum of Biselyngbyolide C (6).



5. Materials and Methods for Evaluation of Anticancer Activity of Biselyngbyolides A & C.

Cell Lines:

The cancer cell lines which were used in this study were HeLa (human cervix adenocarcinoma), HepG2 (human hepatocellular carcinoma) and HCT116 (human colorectal carcinoma). The non-cancerous cell line used was HEK293T (human embryonic kidney).

Cell Culture:

HeLa, HepG2 and HCT116 cell lines were cultured in DMEM with 10% FBS and penicillin/streptomycin. All the cell lines were maintained at 37 °C in 5% CO_2. The cells were cultured at a density that allowed cell division throughout the course of the experiment.

Cell lysis and Immunoblotting:

After treatment of the cells for the stipulated time-points, cells were rinsed with ice-cold PBS before lysis in buffer containing 50mM Tris-HCl (pH 7.4), 100mM NaCl, 1mM EDTA, 1mM EGTA and 1% Triton X-100 with protease/phosphatase inhibitor mixture. The soluble fractions of cell lysates were isolated by centrifugation at 20 000 r.c.f. for 20 min at 4 °C. Samples of the cellular lysates containing an equal amount of proteins were resolved by SDS-PAGE, transferred to PVDF membrane (Millipore) and probed with PARP1, cleaved caspase 3 and cleaved caspase 8 antibodies. β-Actin antibody was used as loading control. Proteins were then visualized with Clarity Max Western^{TM} ECL substrate (Bio Rad) in Bio Rad ChemiDoc MP Imaging System.

Live Dead Assay and Confocal Microscopy:

The polyanionic dye calcein is well retained within live cells, producing an intense uniform green fluorescence in live cells (ex/em ~495 nm/~515 nm). EthD-1 enters cells with damaged membranes and undergoes a 40-fold enhancement of fluorescence upon binding to nucleic acids, thereby producing a bright red fluorescence in dead cells (ex/em ~495 nm/~635 nm). EthD-1 is

excluded by the intact plasma membrane of live cells. Briefly, cells were seeded in grooves of confocal dishes, treated with specific concentrations of synthesized compounds and incubated for 16h at 37°C. Henceforth, the media was discarded and the cells were carefully washed with ice cold 1x PBS. The fluorochrome solution was prepared by adding 1µL of Calcein-AM (2µM) and 0.2µL Ethidium homodimer 1 (0.2µM) to 2mL PBS. 200µL of the fluorochrome solution was added to the groove of the confocal dish. The confocal dish then incubated for 40 minutes at room temperature, protected from light. After incubation, cells were mildly washed and imaged using Olympus Confocal microscope in the wavelength range of FITC (Fluorescein Isothiocyanate) (495/519 nm) and Rhodamine-Red X (572/591 nm). Graphical representation of the percentage of dead cells vs. treatment dosage was generated using GraphPad Prism software.

Study of Cell Growth by Phase Contrast Microscopy:

The previously described cancerous and non-cancerous cells were seeded in 6-well plates, treated with synthesized biselyngbyolides A and C and incubated for 16 h at 37°C. Images were captured using Zeiss Axiovert 40 CFL Trinocular Inverted Fluorescence Phase Contrast Microscope, one set at 0^{th} hour and the other at 16^{th} hour post compound treatment. The scale bars were of 100 µm length and inserted using ImageJ software.

In vitro MTT Assay for Cytotoxicity:

The MTT assay is used to measure cellular metabolic activity as an indicator of cell viability, proliferation and cytotoxicity. This colorimetric assay is based on the reduction of a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or MTT) to purple formazan crystals by metabolically active cells. The viable cells contain NAD(P)H-dependent oxidoreductase enzymes which reduce the MTT to formazan. Exponentially growing cells were seeded at 1×10^4 cells per well in 96-well plates, treated with varying concentrations of

the synthesized compounds and incubated for 24 h. 50 µL of MTT (5 mg/ml) was added to each well and the plates were incubated for 3 h at 37 °C. The supernatant was discarded and then the formazan product was dissolved by adding 100 µl DMSO to each well. The MTT absorbance value was detected at 590 nm in a ThermoScientific Multiscan Go microplate spectrophotometer. Cells treated with Rotenone was used as positive control of cancer cell death, whereas the untreated cells were considered to be negative control. Data was analysed as percentage of viable cells vs. dosage of treatment. IC50 values were obtained using GraphPad Prism software. Each synthesized compound was tested in triplicate in at least two independent experiments.

Figure S3: Phase contrast microscopy of HeLa and HepG2 cell lines following 16 h treatment of synthesized compounds biselyngbyolides A (**4**) and biselyngbyolides C (**6**).