Supporting Information

Synthesis of the fungal macrolide berkeleylactone A and its inhibition of microbial biofilm formation

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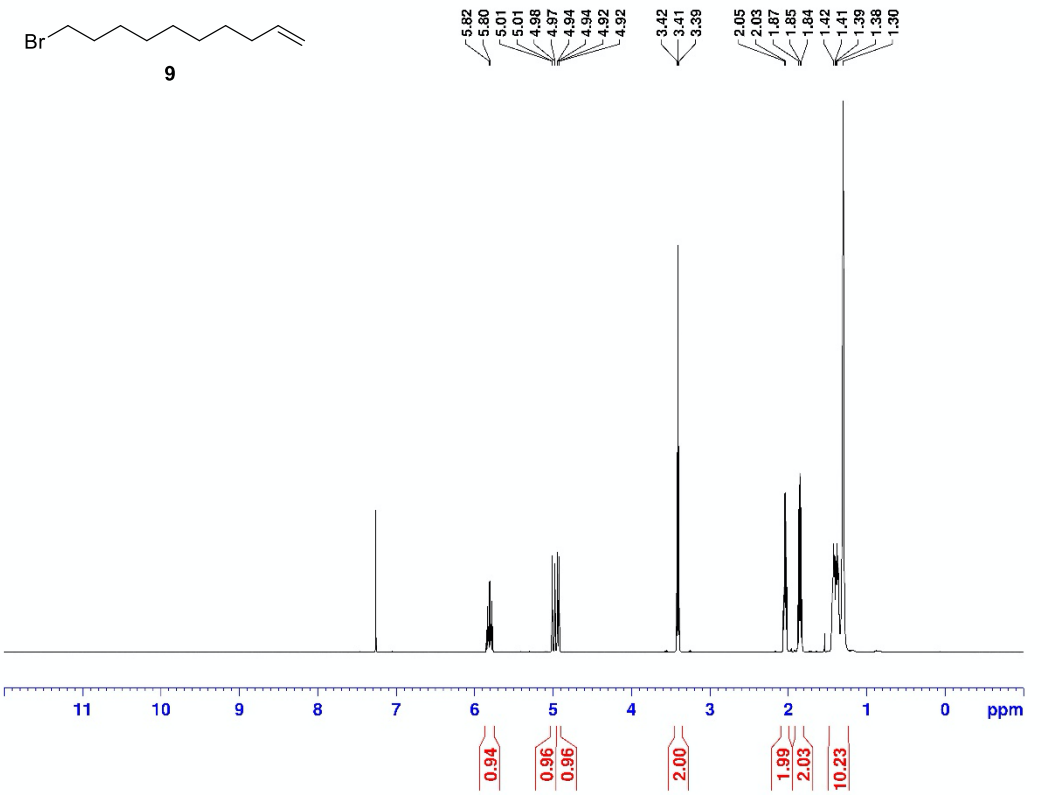
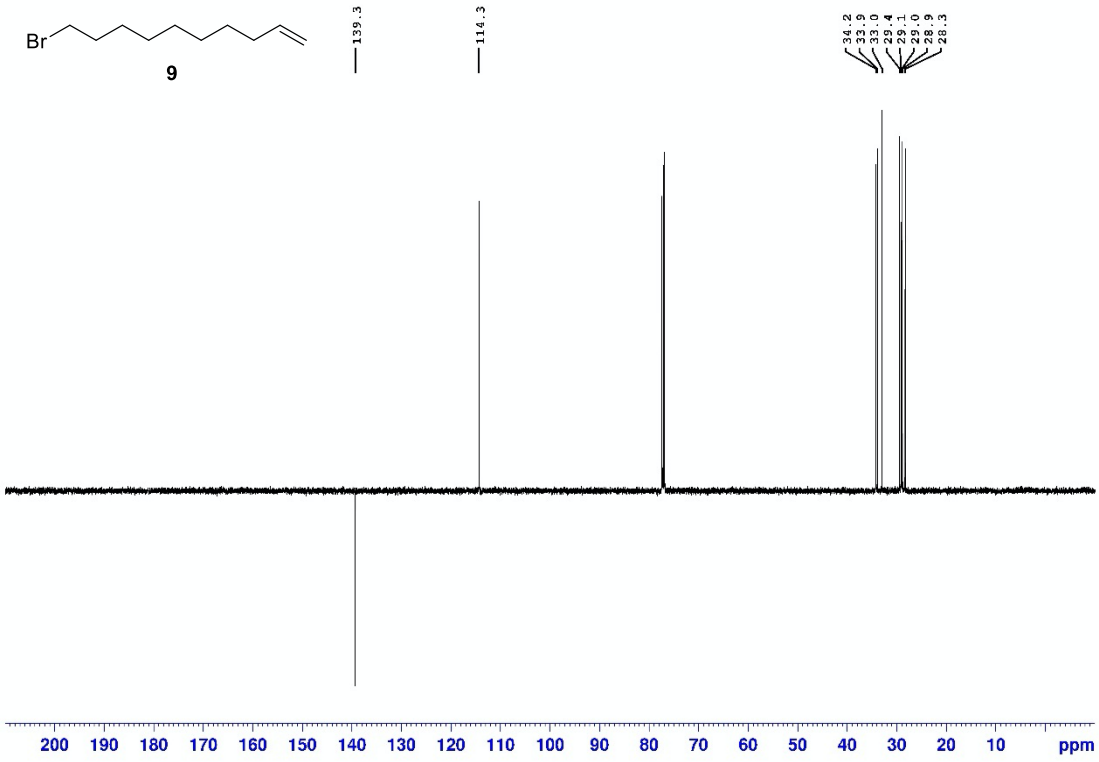
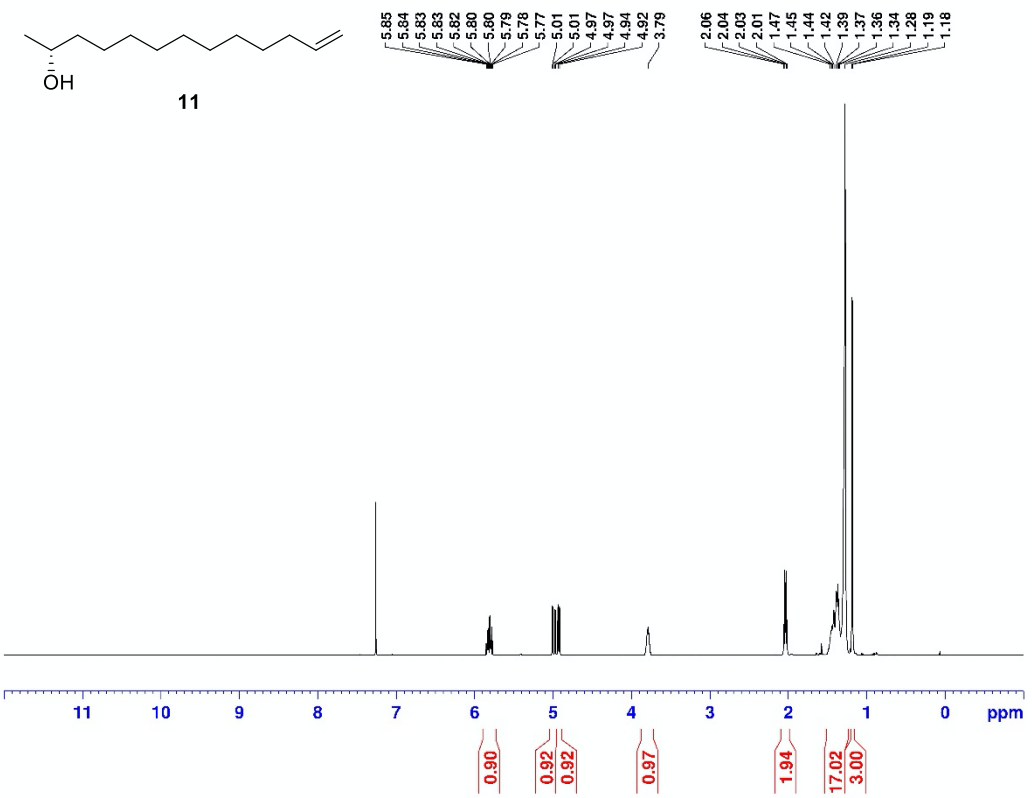
Table of contents **page**



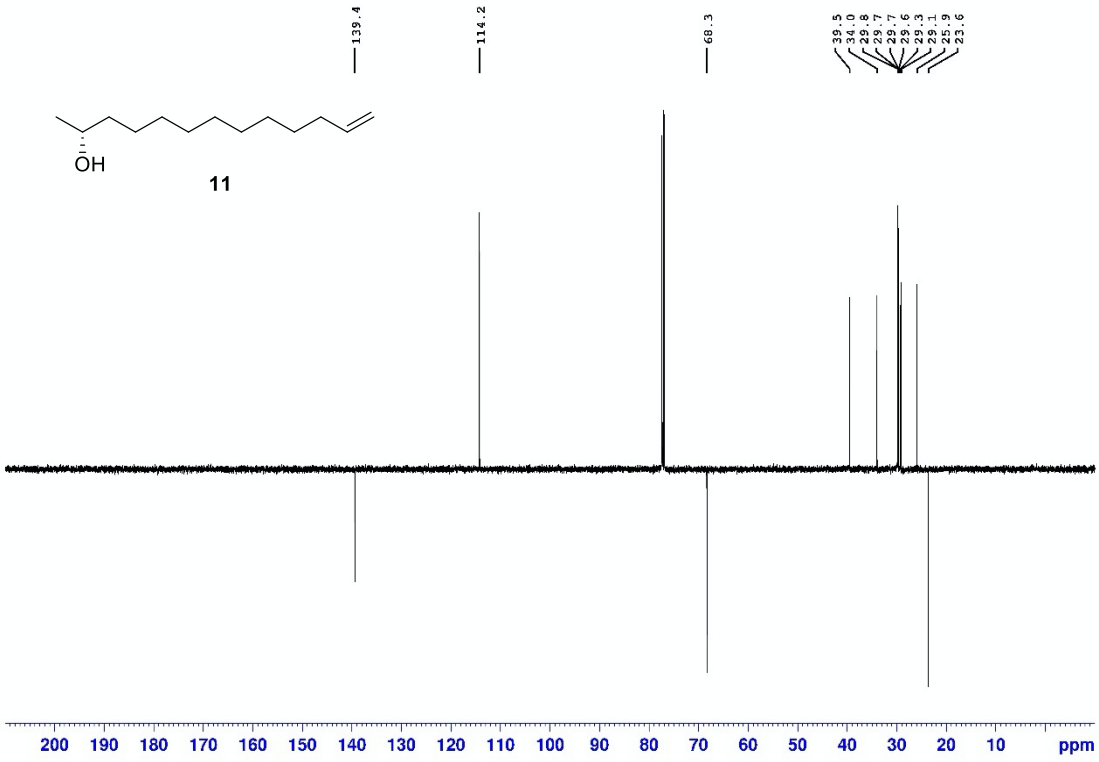
^{1}H-NMR spectrum of compound **9** in $CDCl_3$.



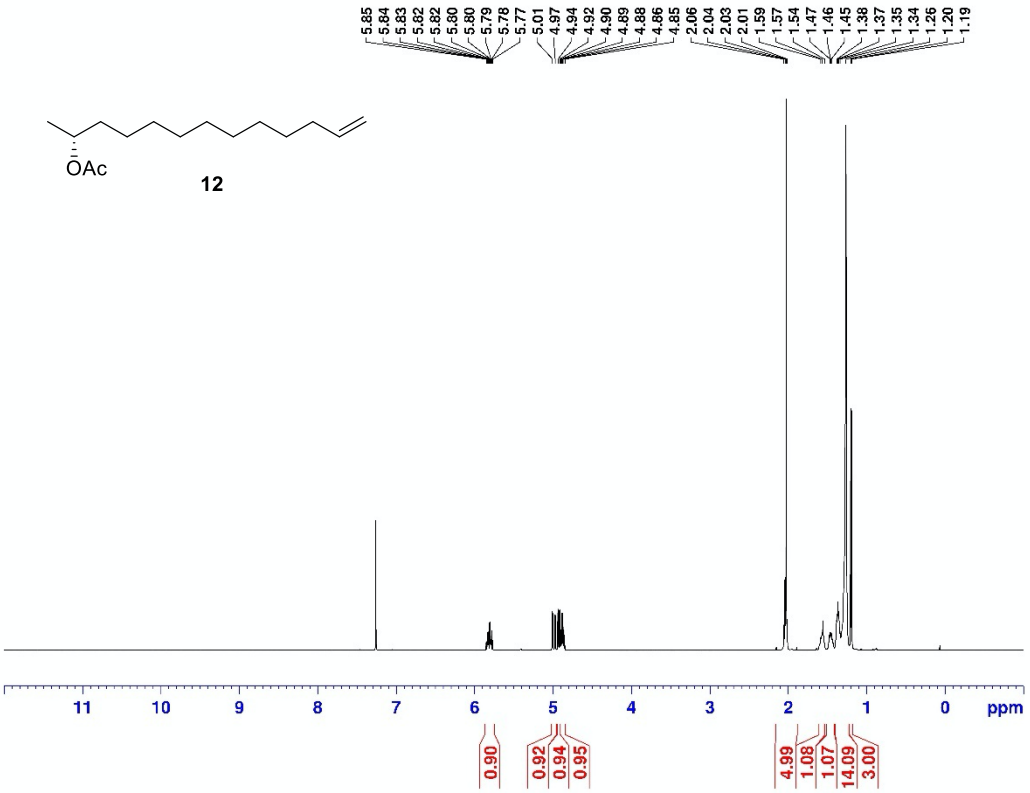
^{13}C-NMR spectrum of compound **9** in $CDCl_3$.



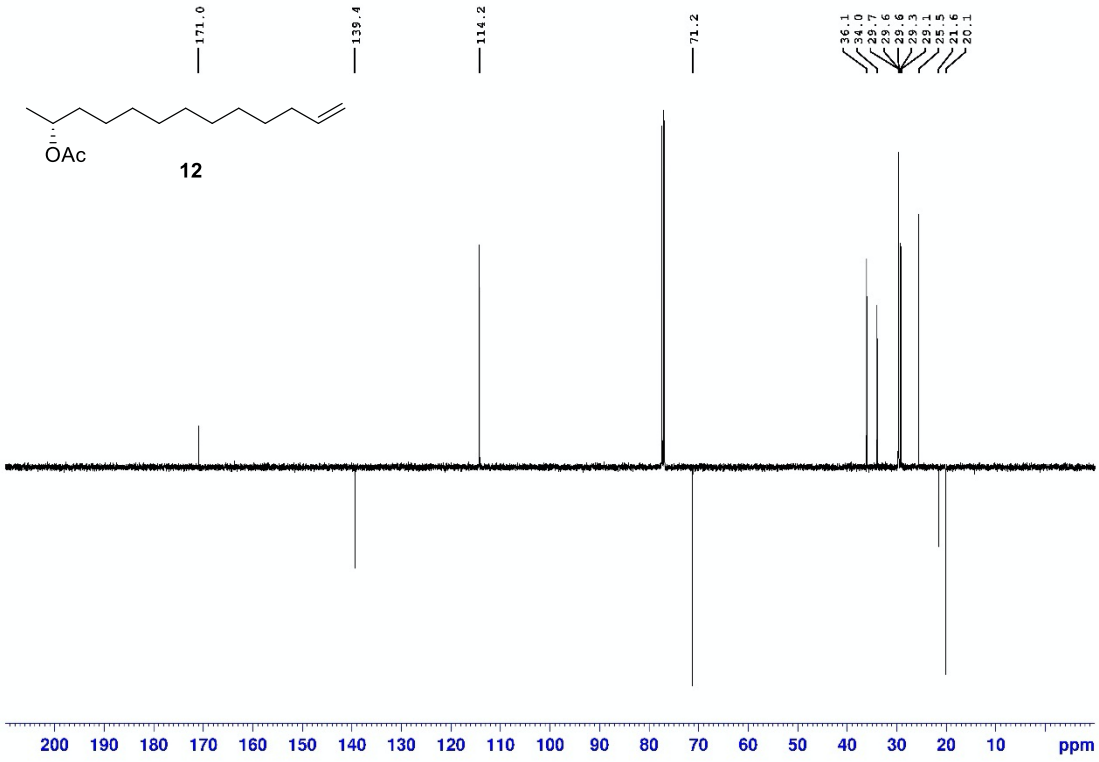
^{1}H-NMR spectrum of compound **11** in $CDCl_3$.



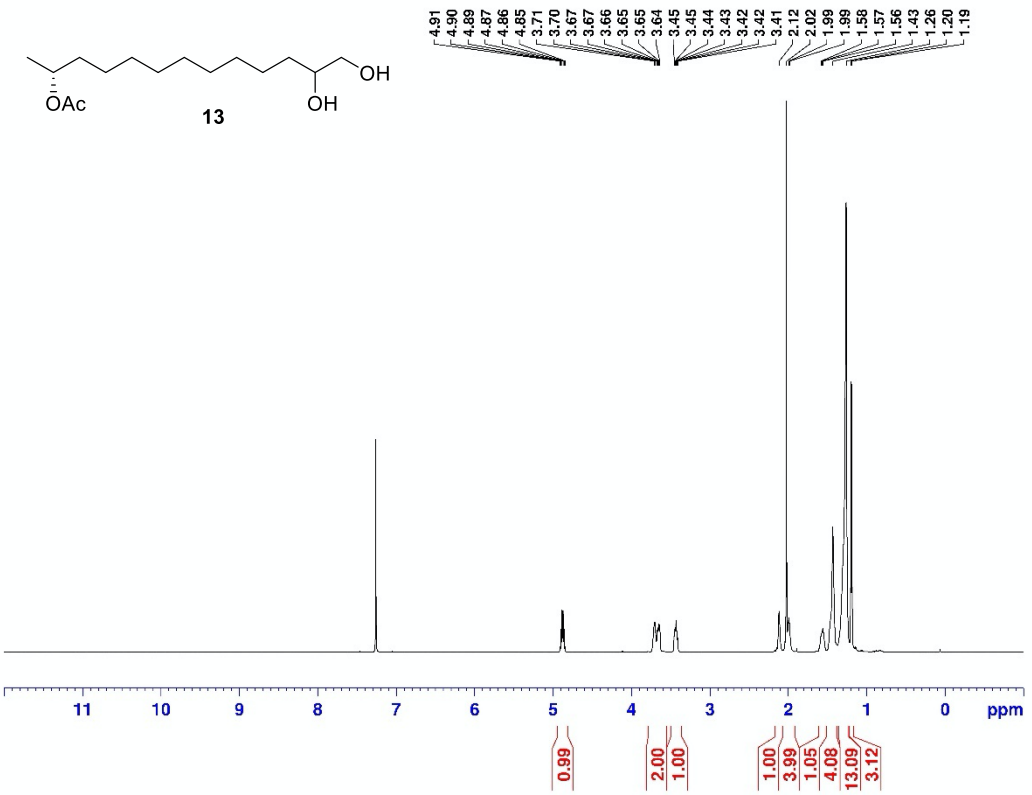
^{13}C-NMR spectrum of compound **11** in $CDCl_3$.



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



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



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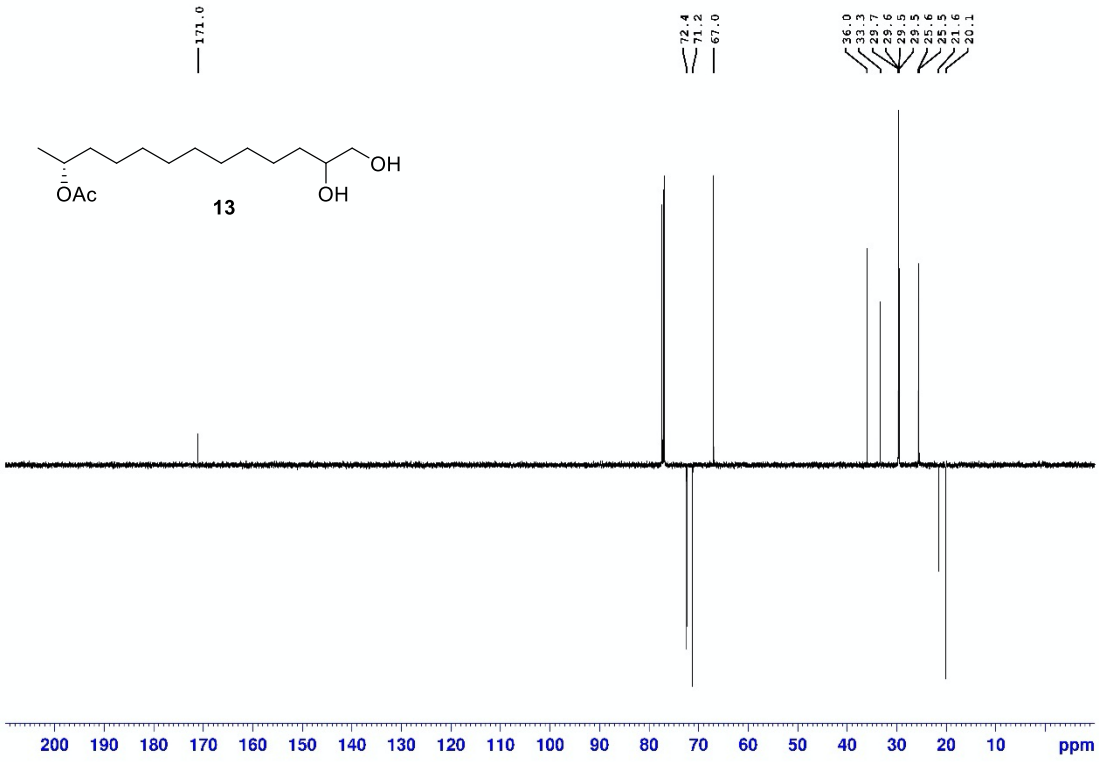
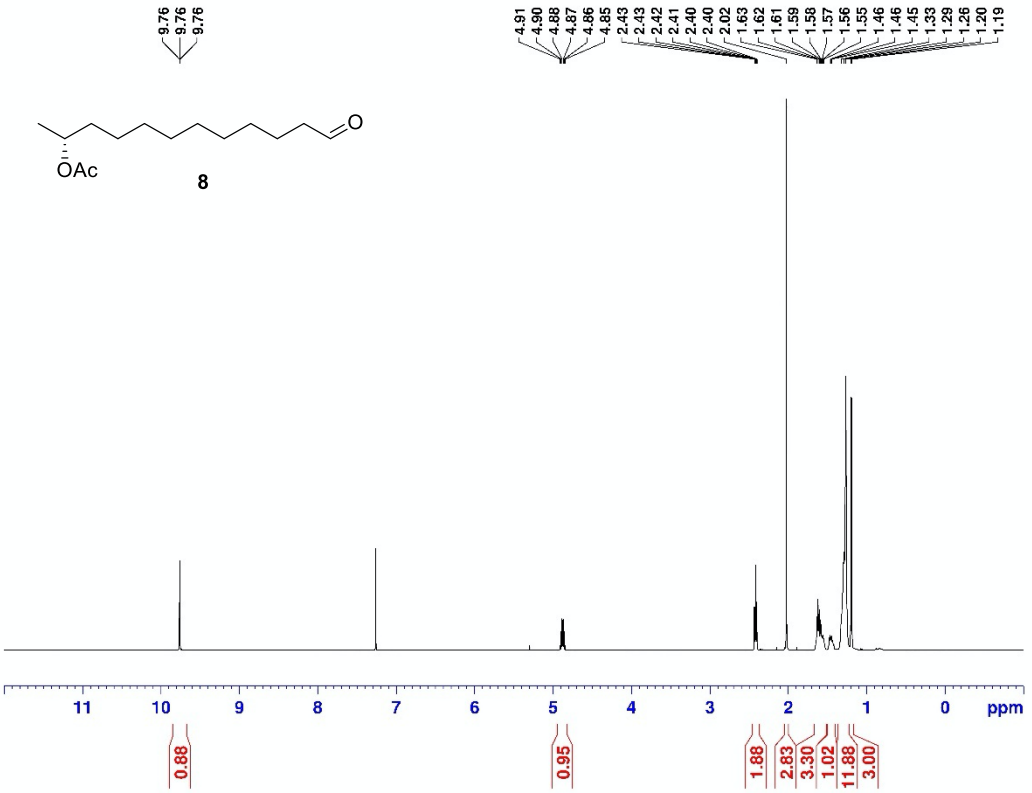


^{13}C-NMR spectrum of compound **13** in $CDCl_3$.



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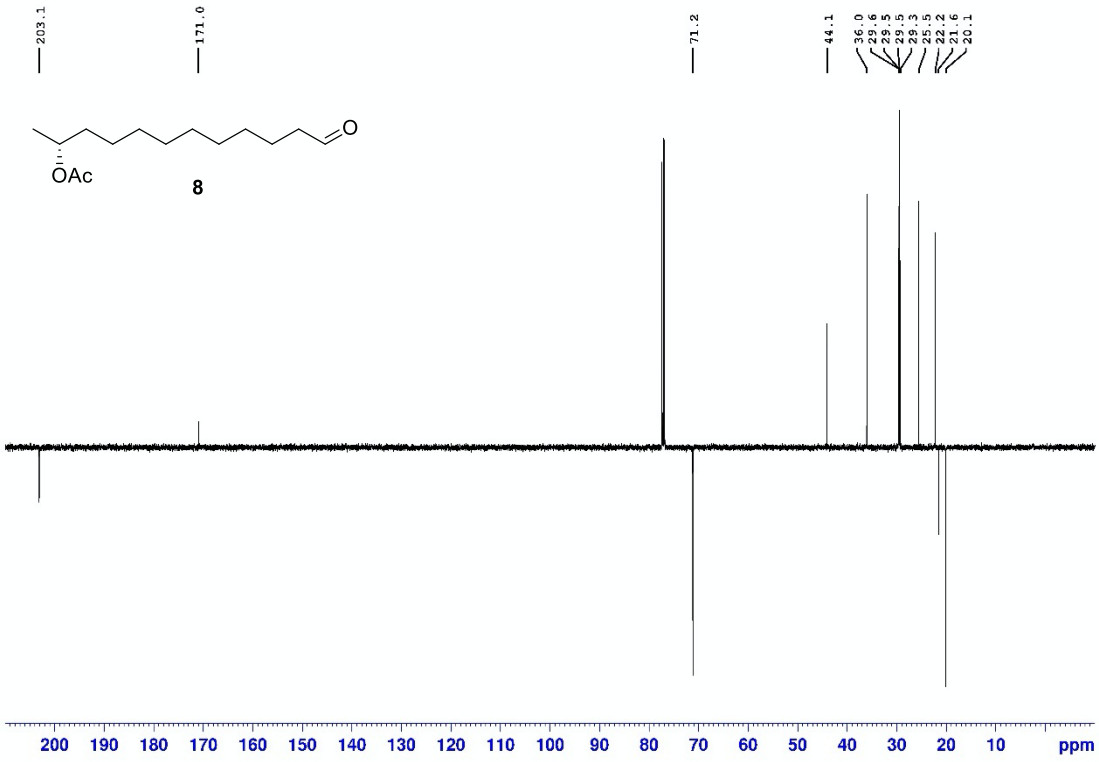

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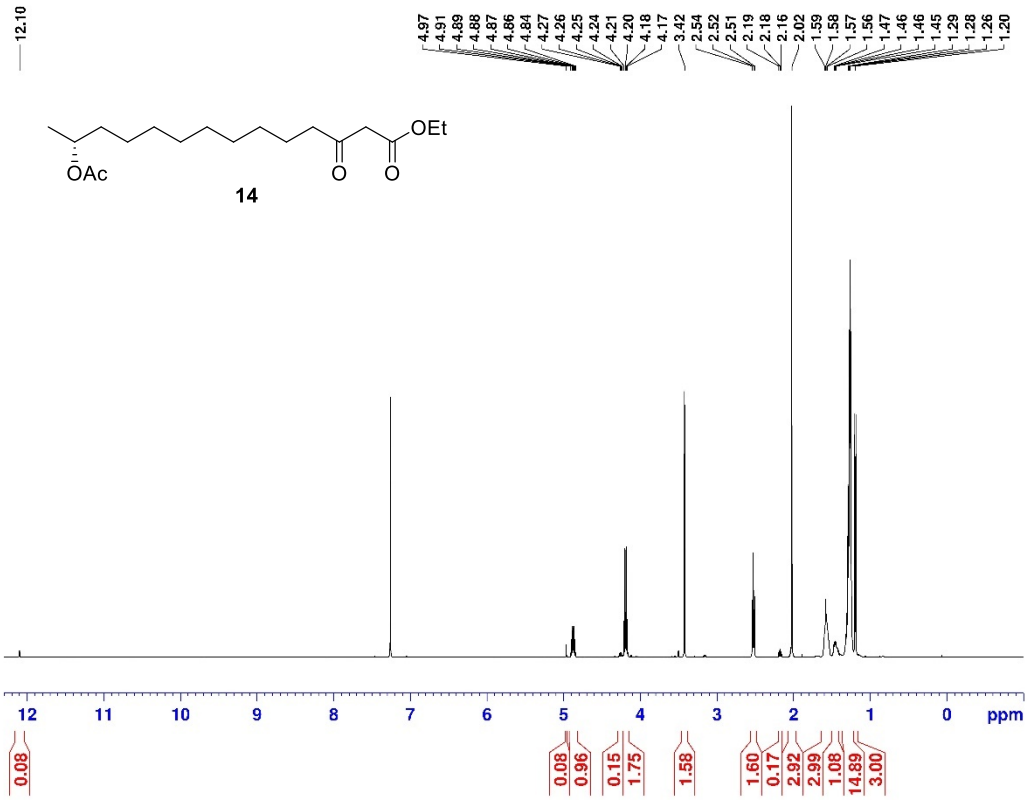

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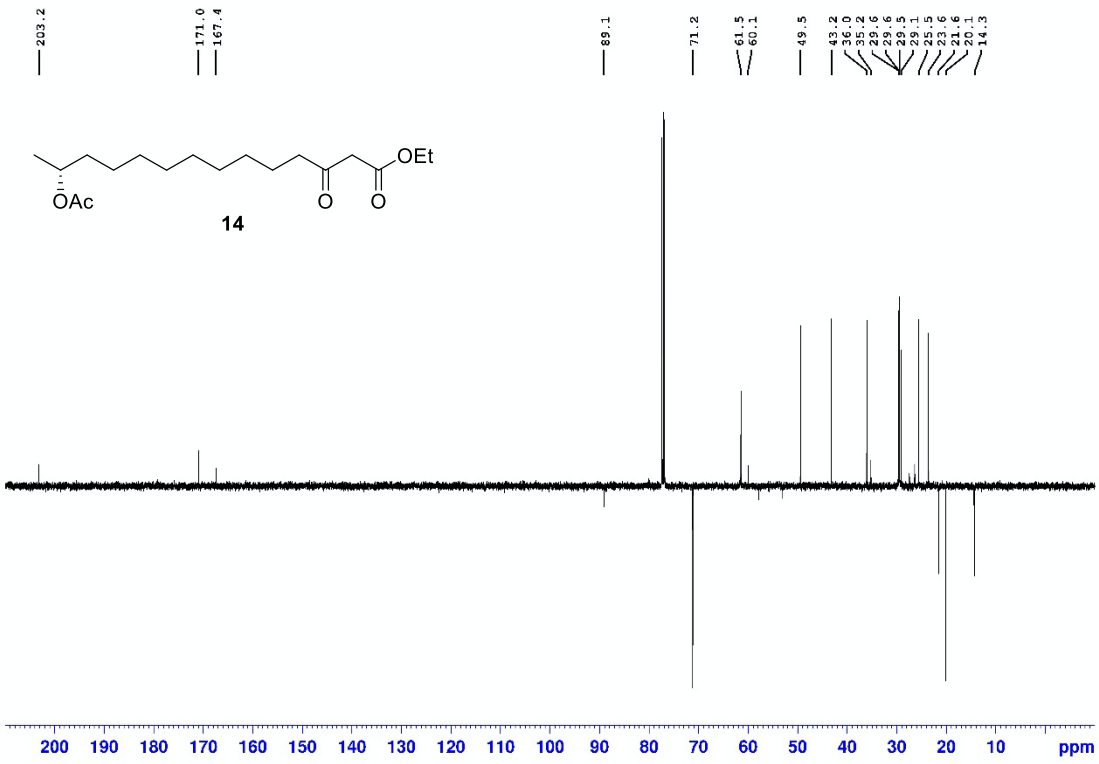
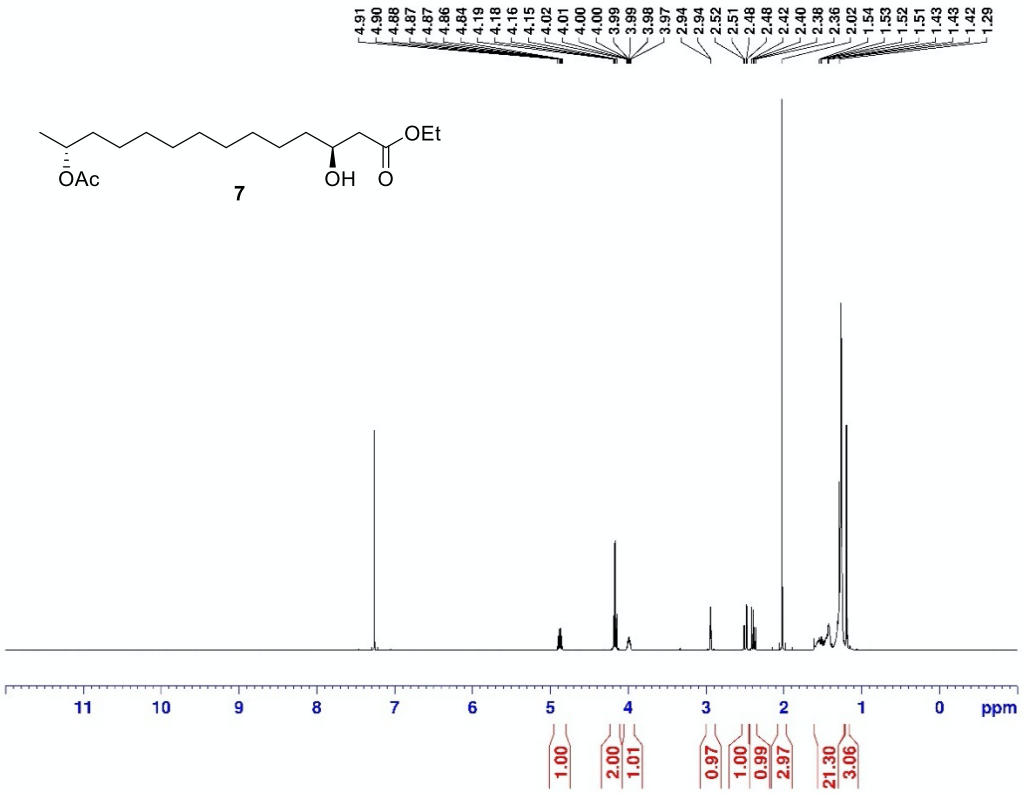


^{13}C-NMR spectrum of compound **14** in $CDCl_3$.



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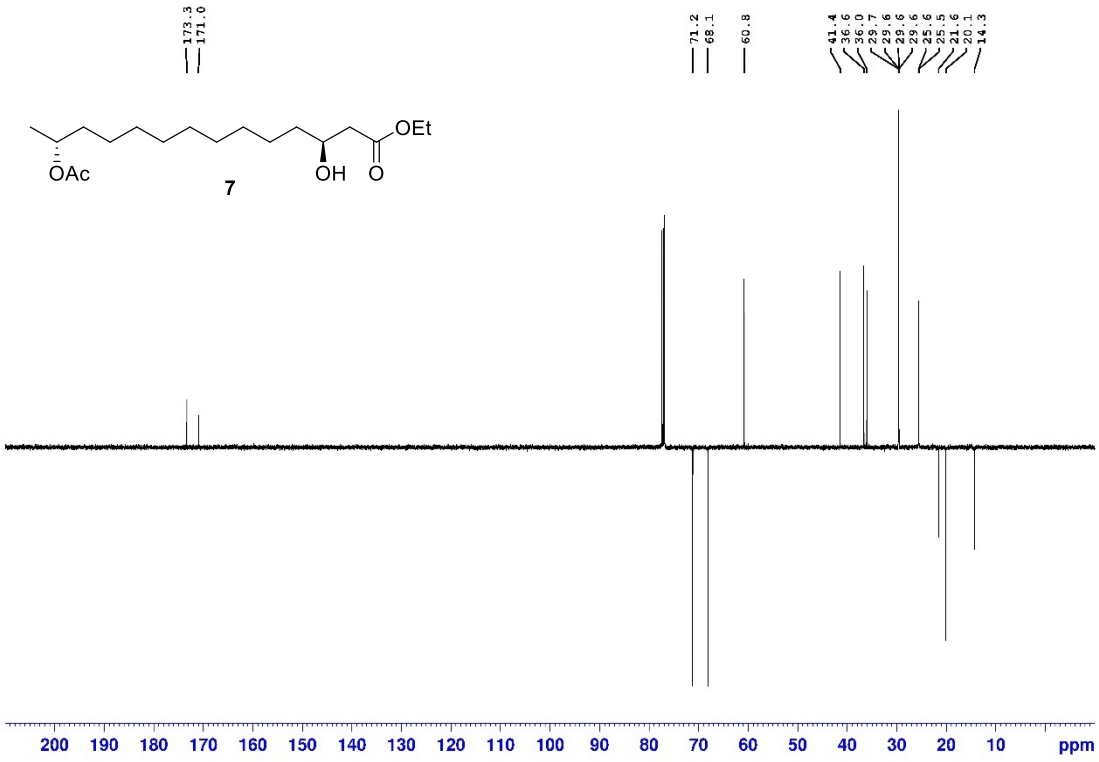
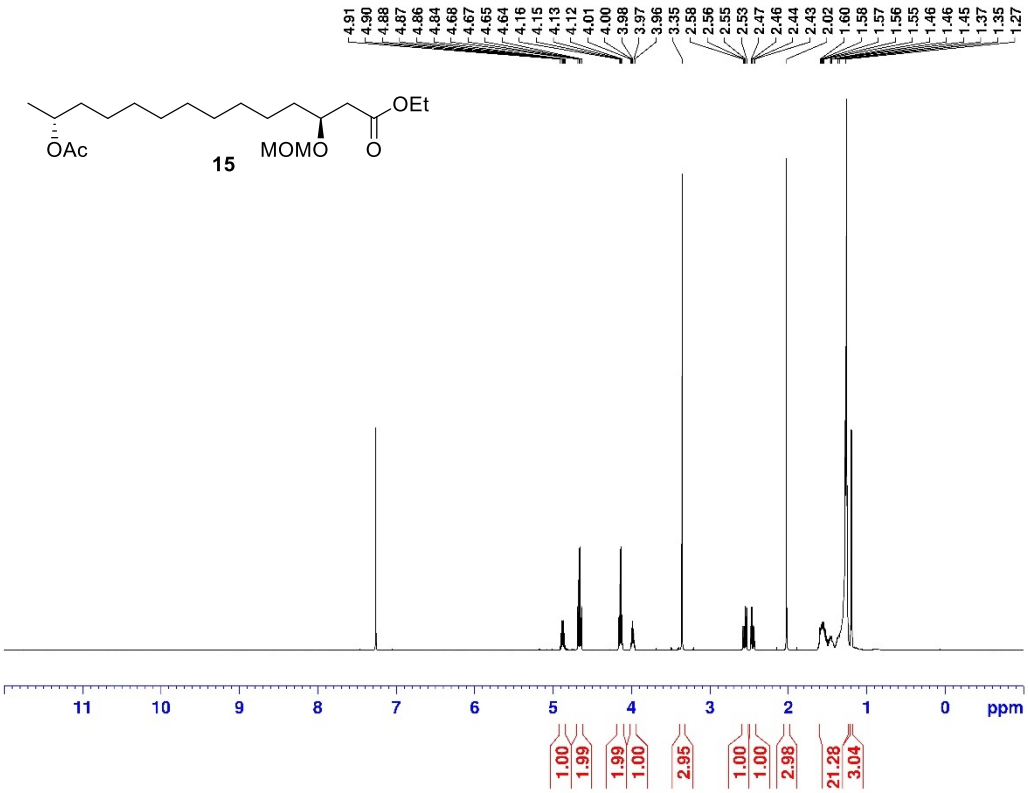
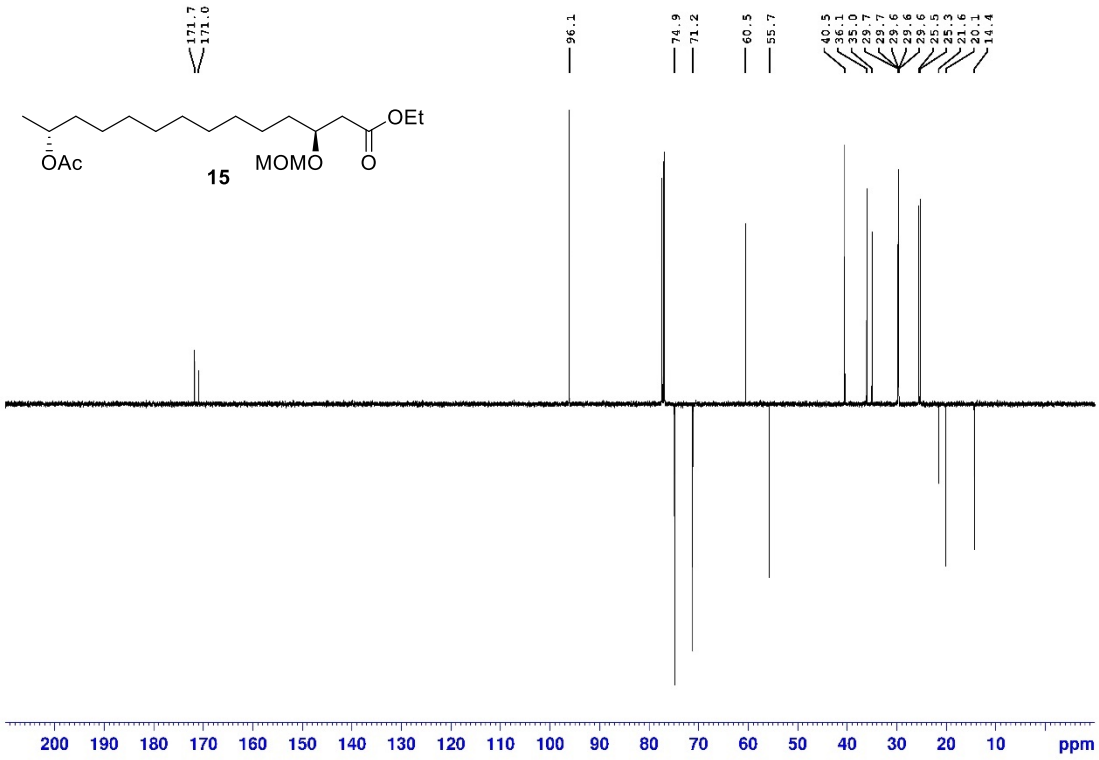
^{1}H-NMR spectrum of compound **7** in $CDCl_3$.



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



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



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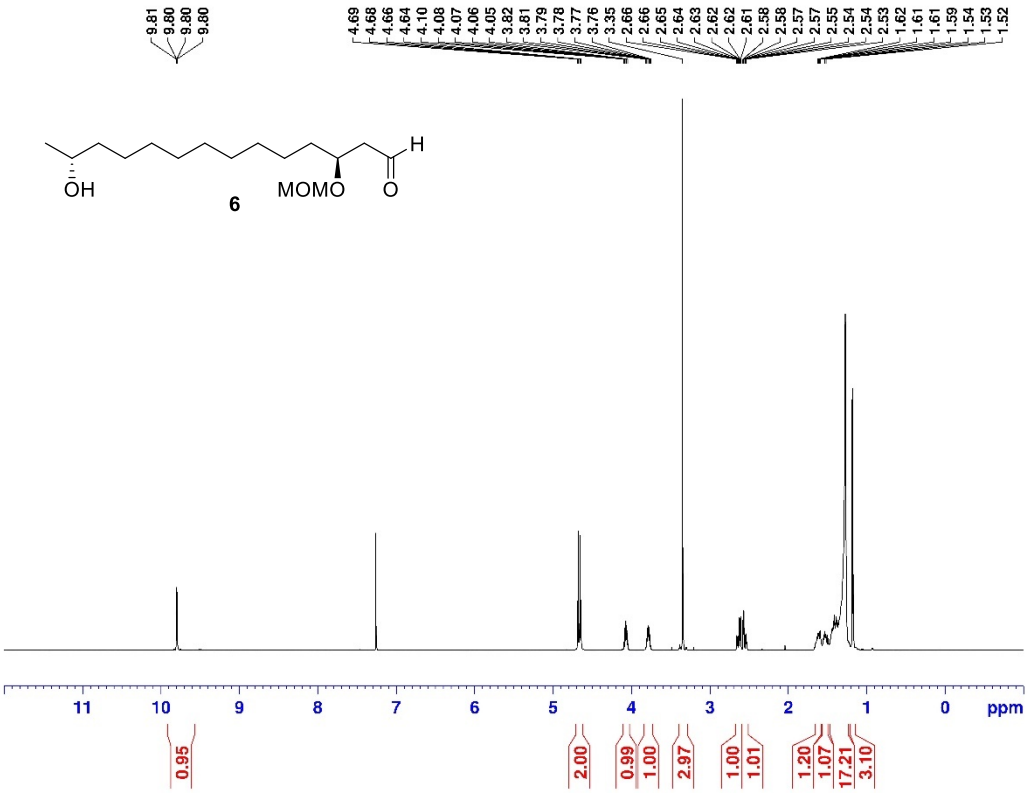

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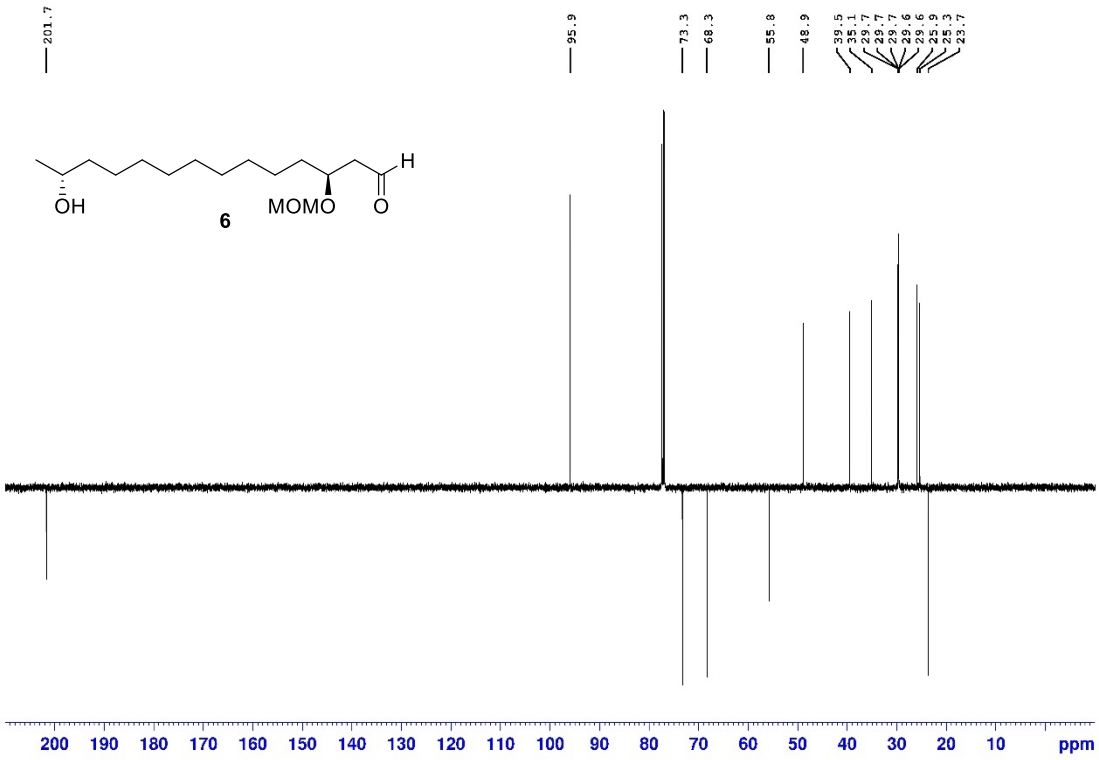

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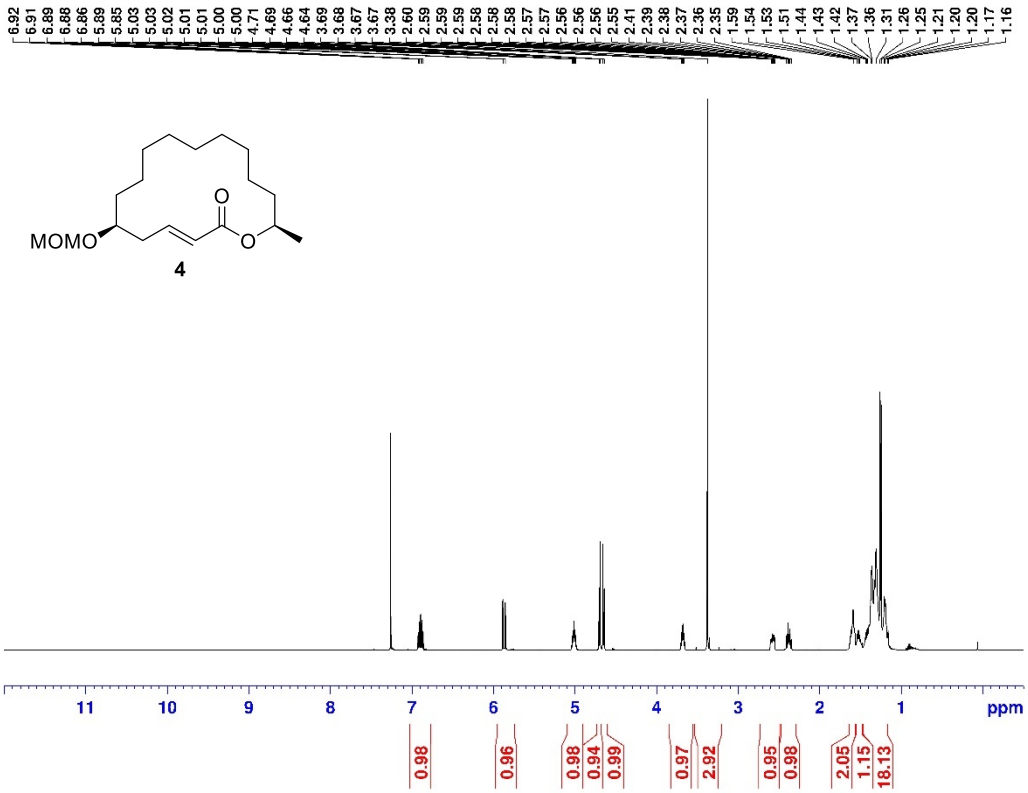

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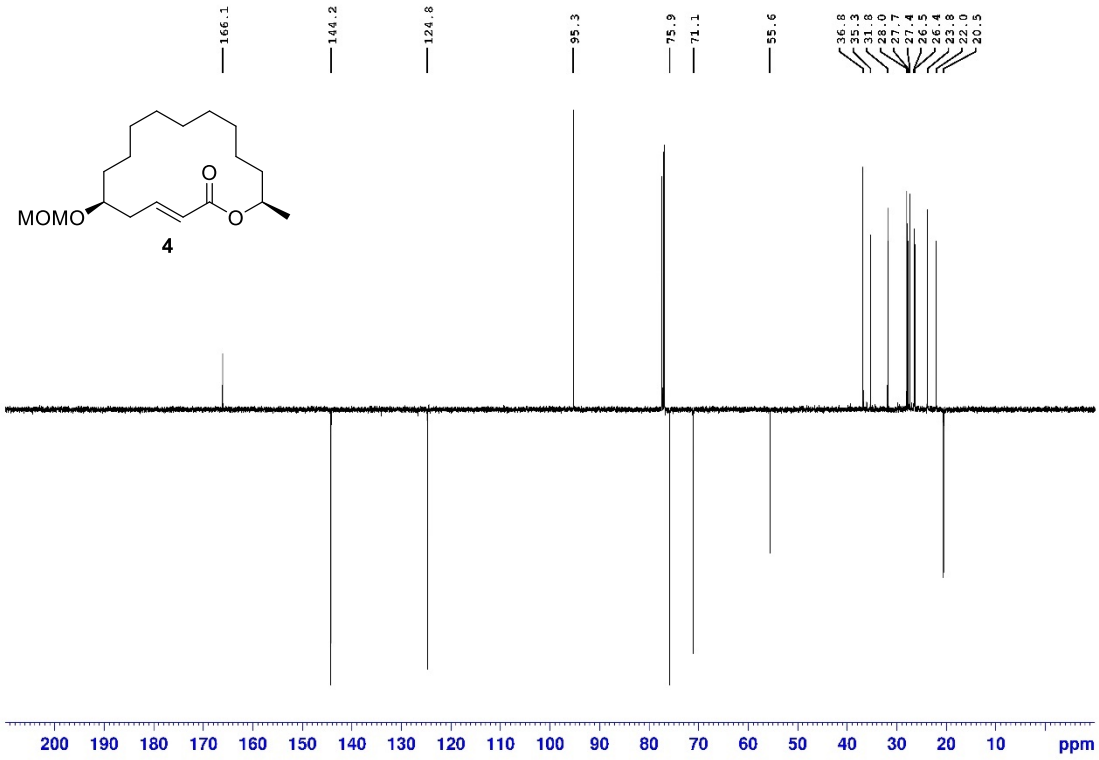
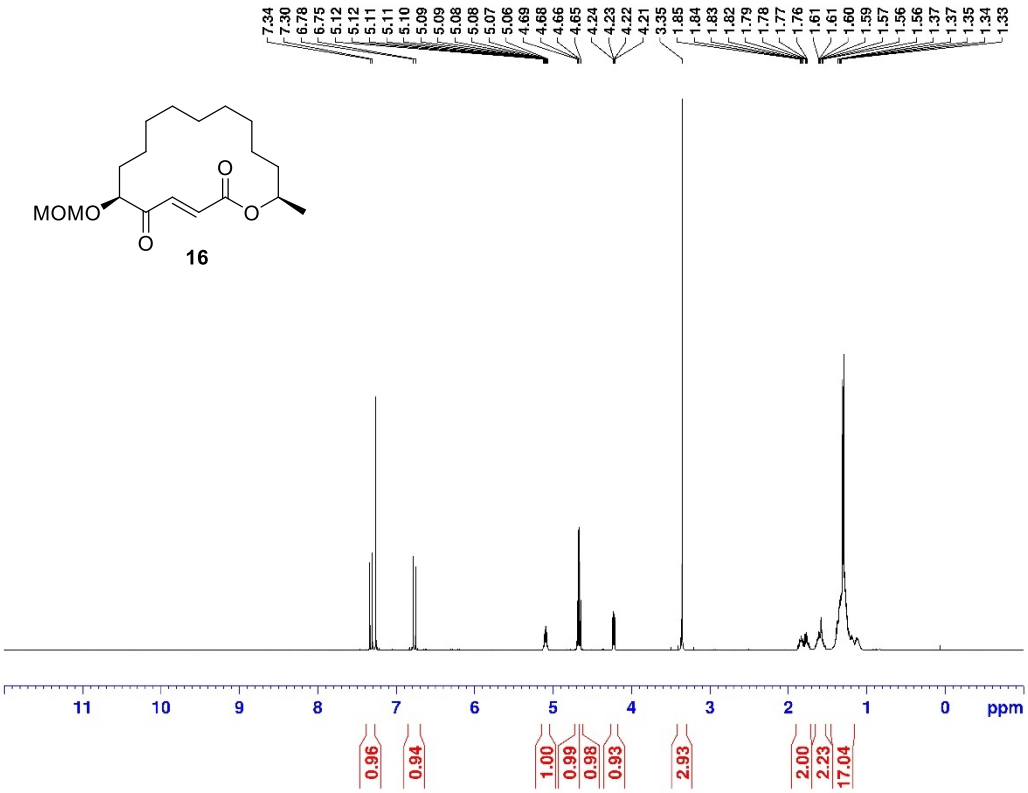
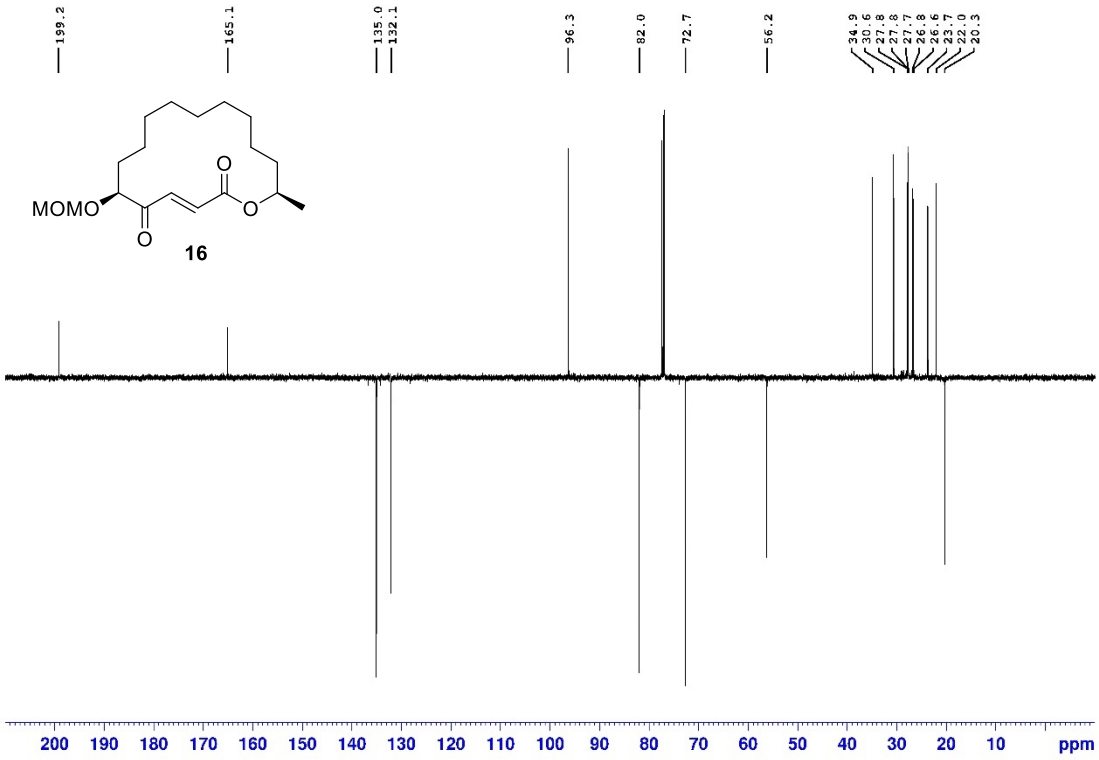


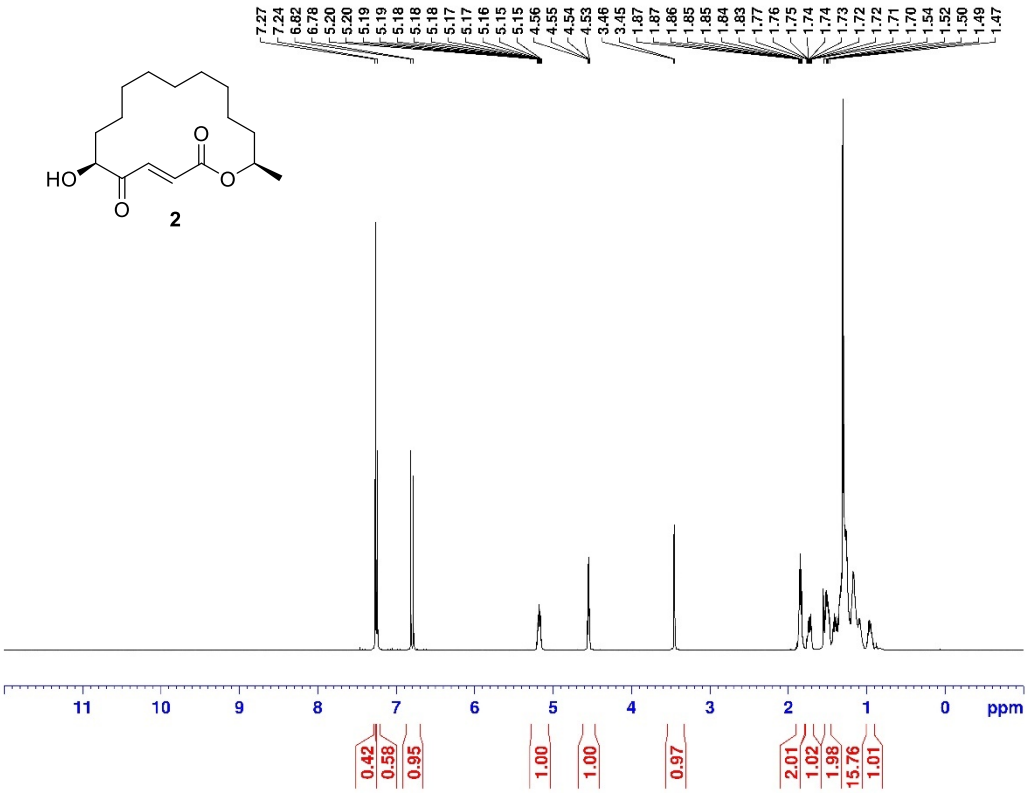
^{13}C-NMR spectrum of compound **4** in $CDCl_3$.



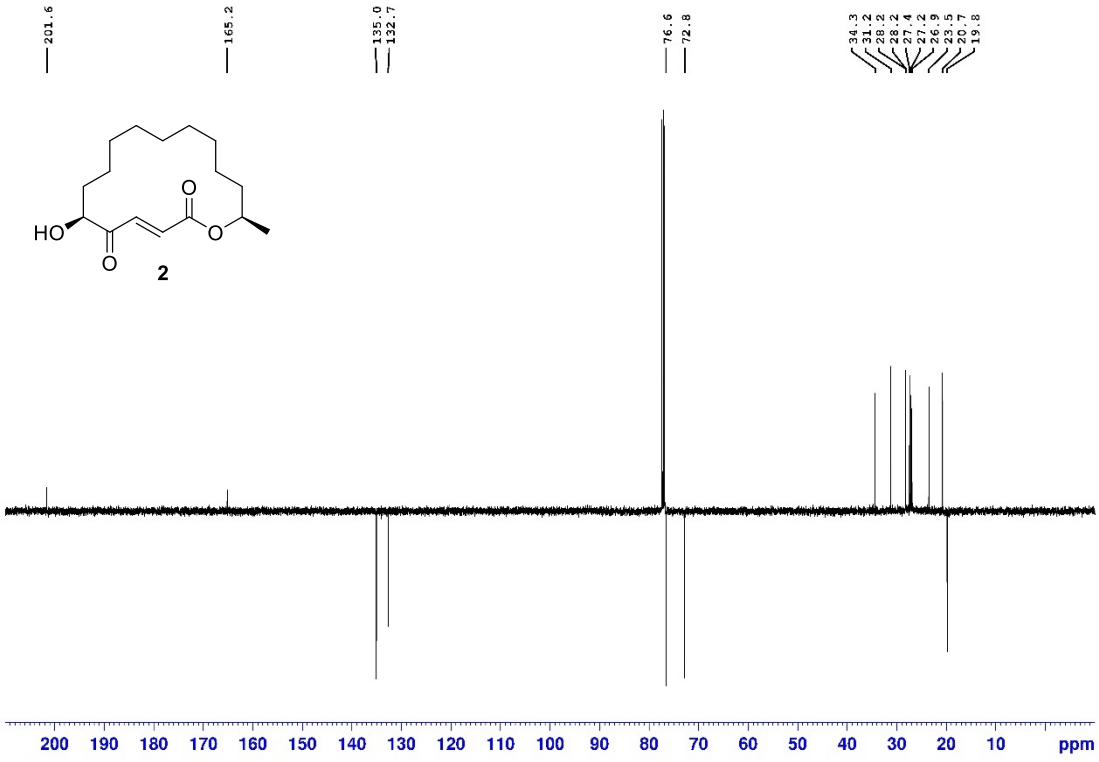
^{1}H-NMR spectrum of compound **16** in $CDCl_3$.



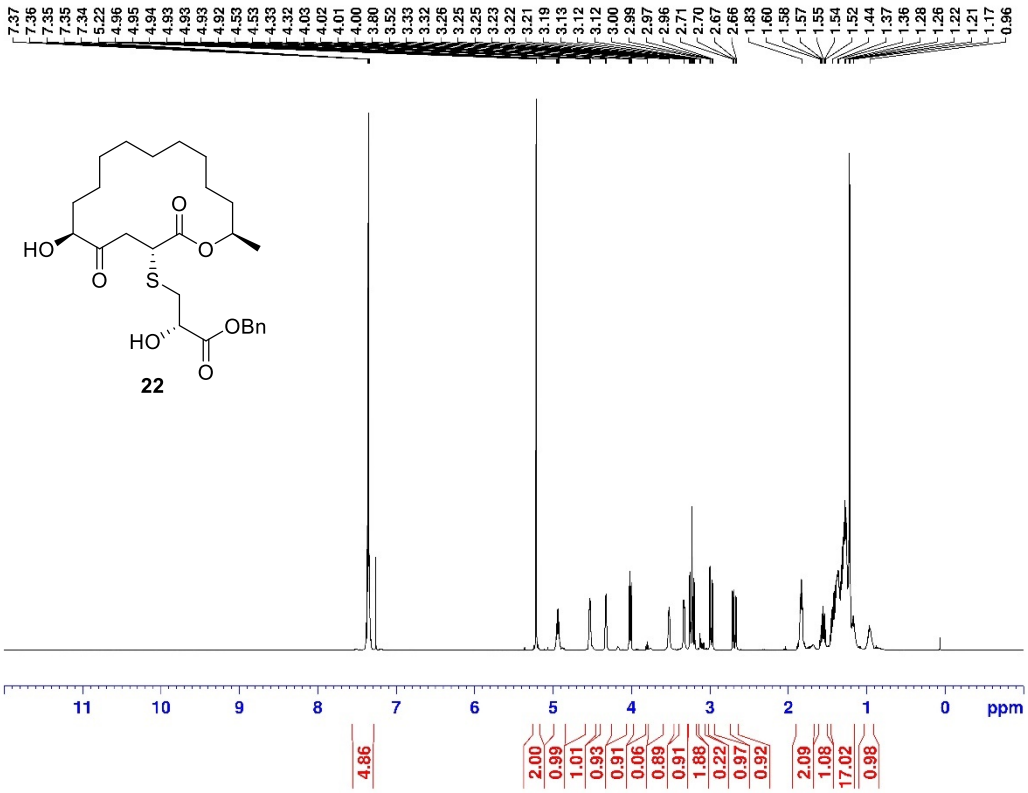
^{13}C-NMR spectrum of compound **16** in $CDCl_3$.



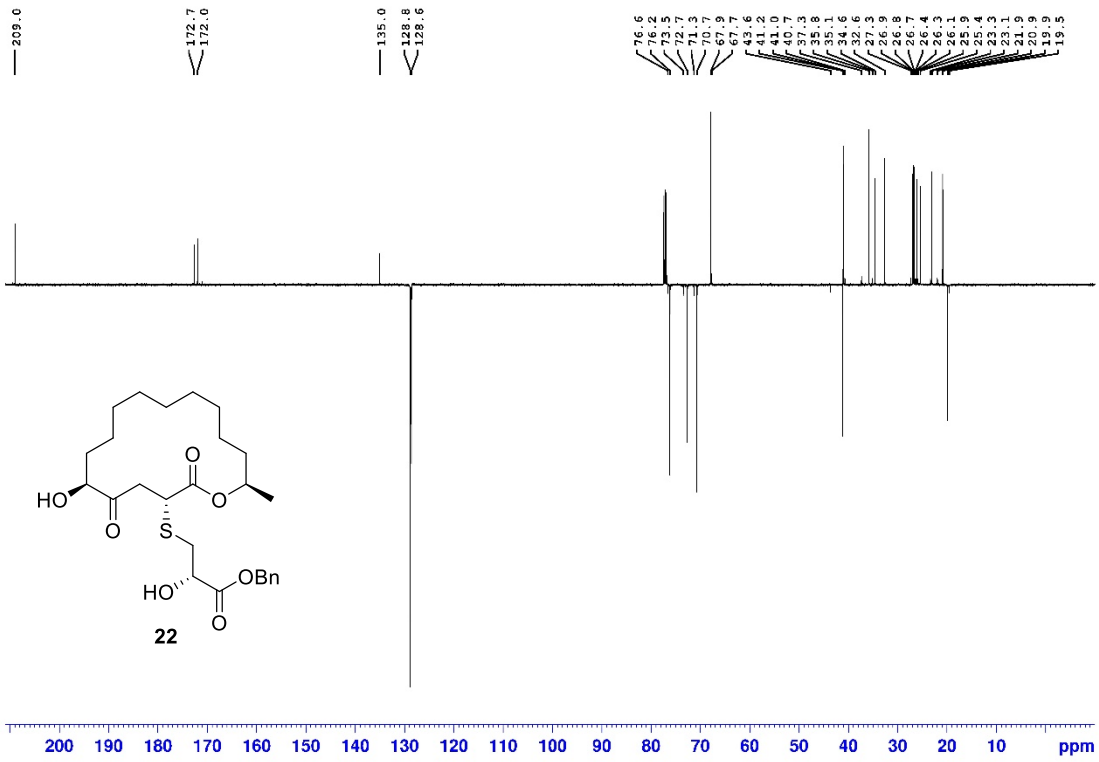
^{1}H-NMR spectrum of compound **2** in $CDCl_3$.



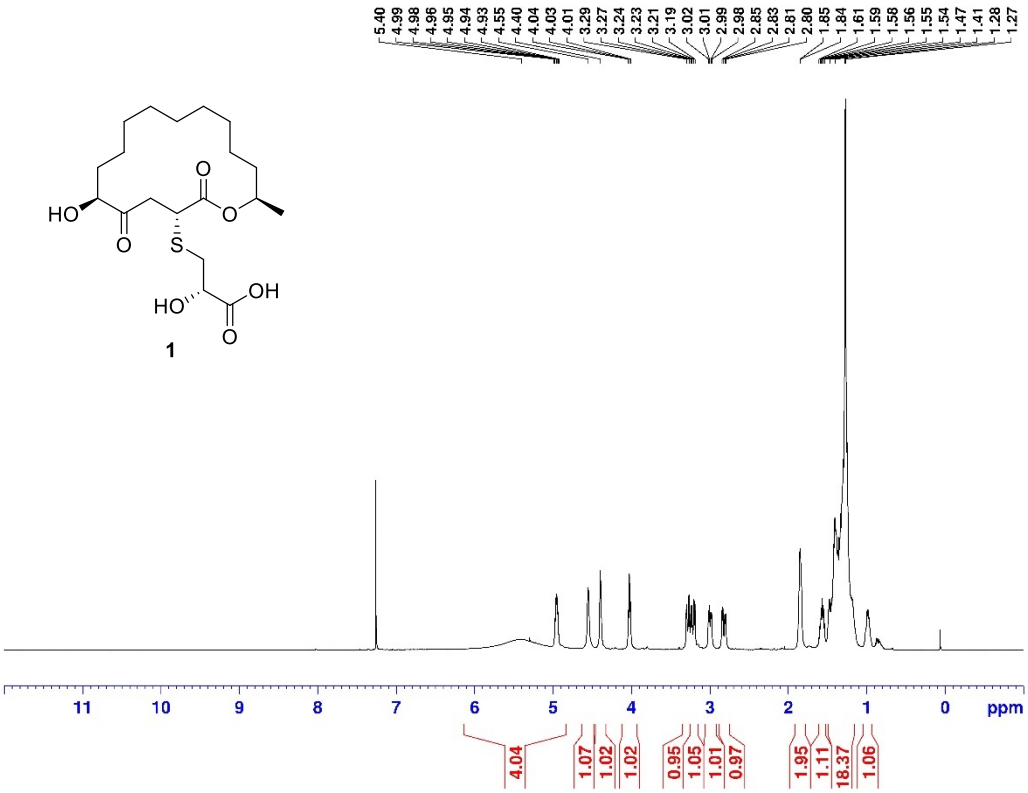
^{13}C-NMR spectrum of compound **2** in $CDCl_3$.



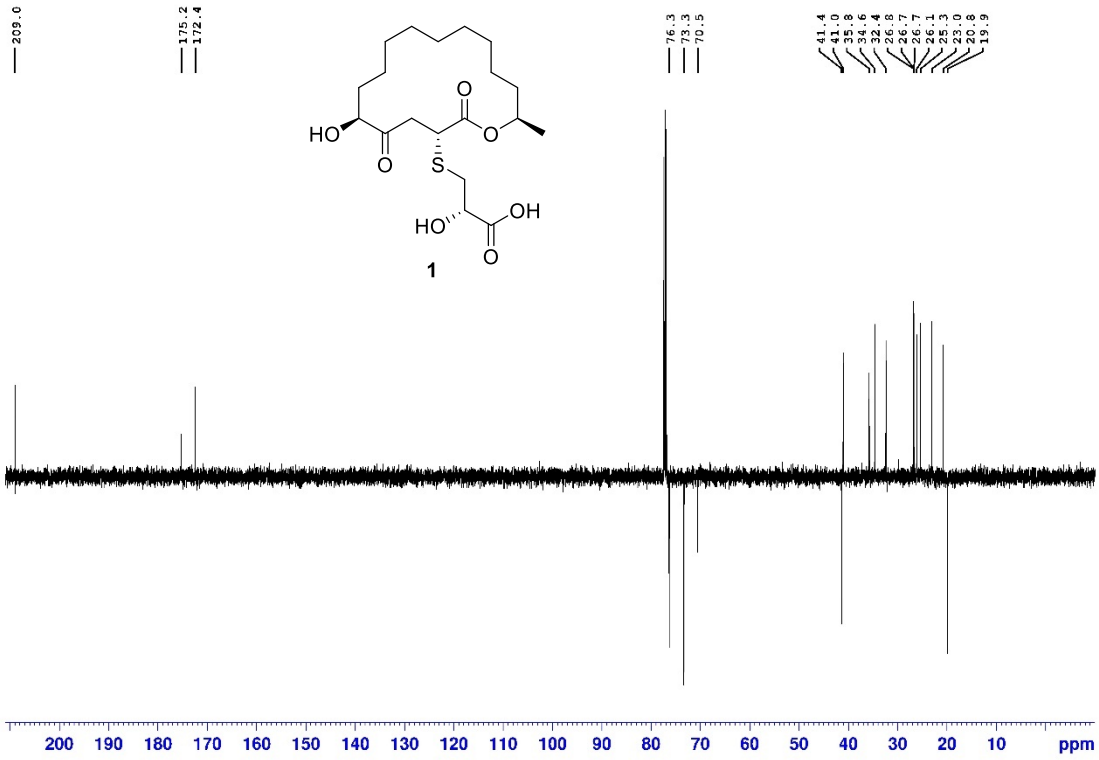
^{1}H-NMR spectrum of compound **22** in $CDCl_3$.



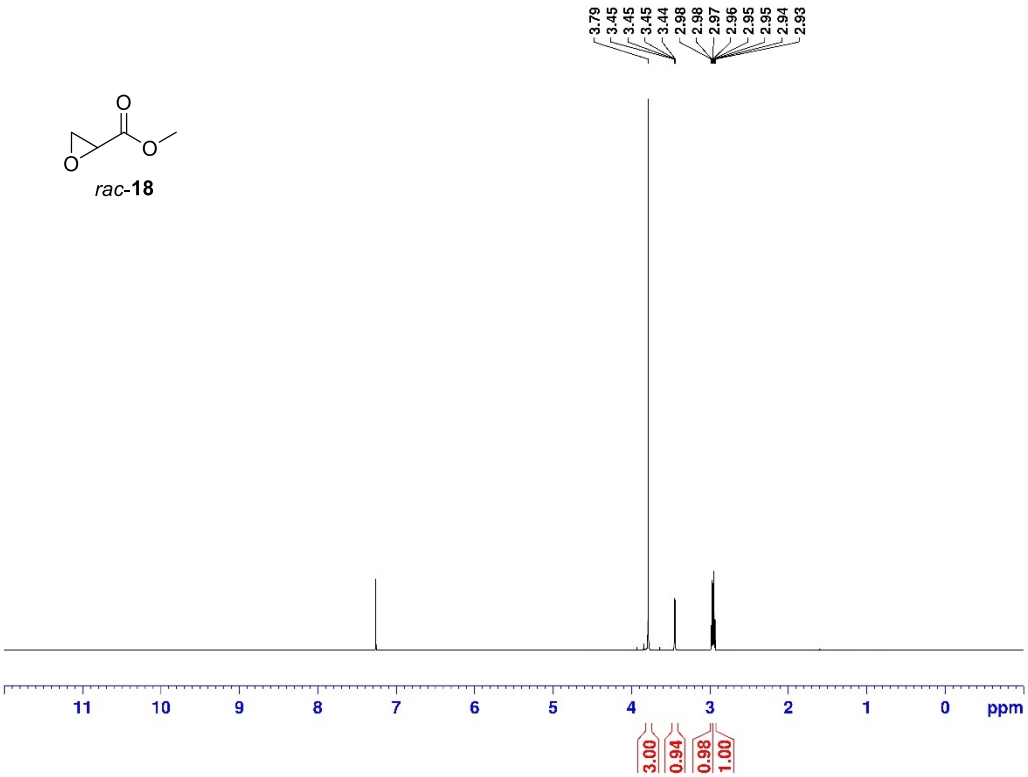
^{13}C-NMR spectrum of compound **22** in $CDCl_3$.



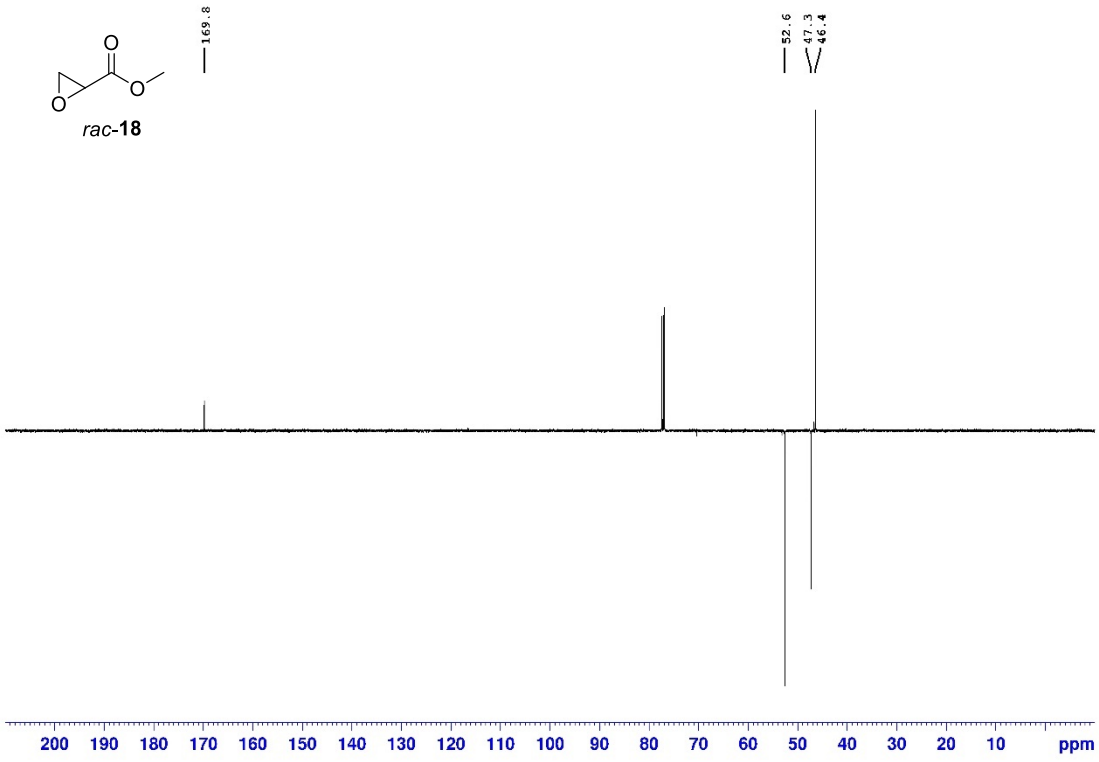
^{1}H-NMR spectrum of compound **1** in $CDCl_3$.



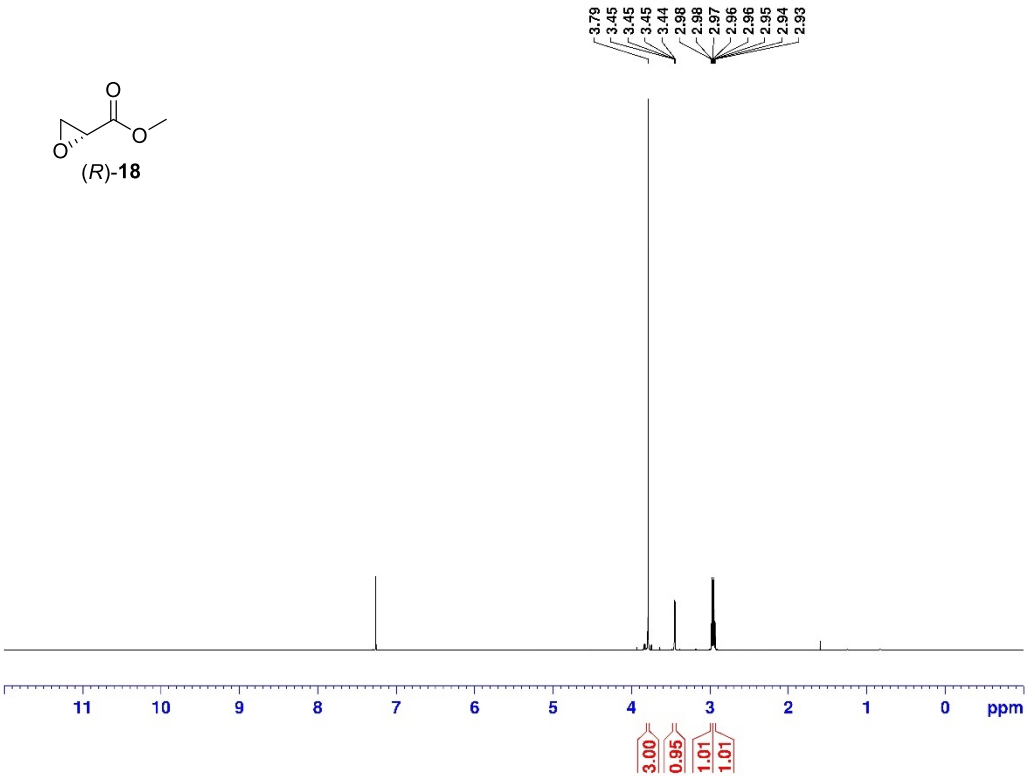
^{13}C-NMR spectrum of compound **1** in $CDCl_3$.



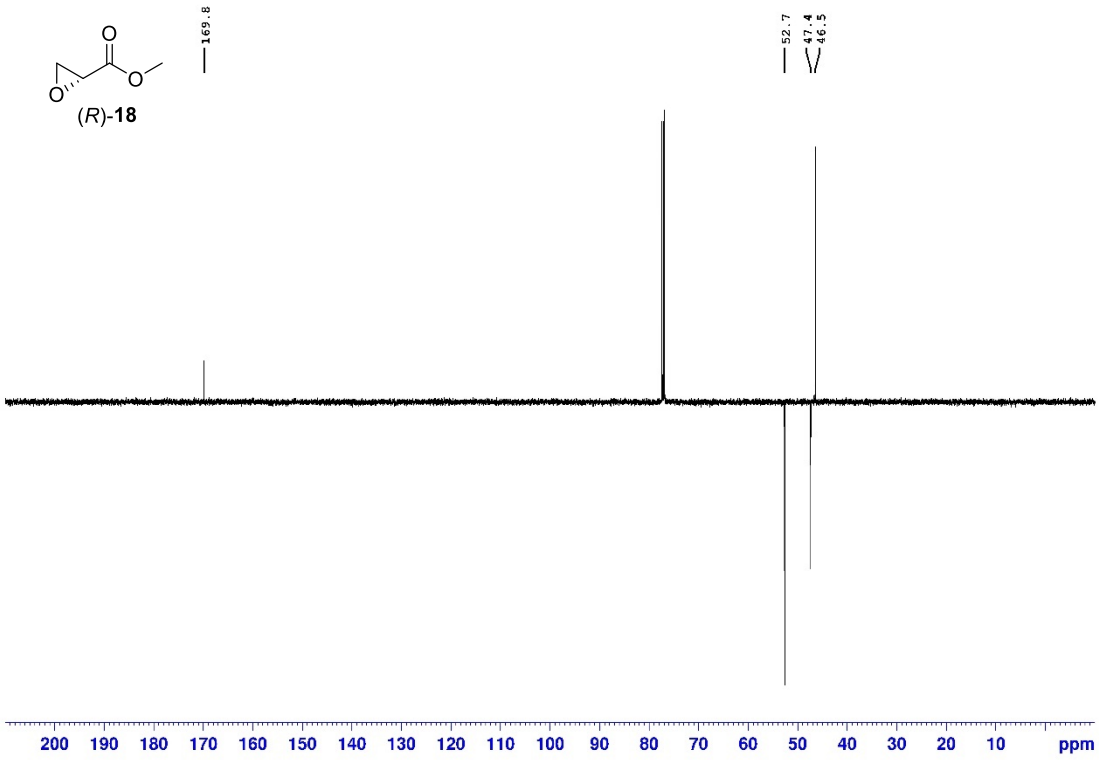
^{1}H-NMR spectrum of compound *rac*-**18** in $CDCl_3$.



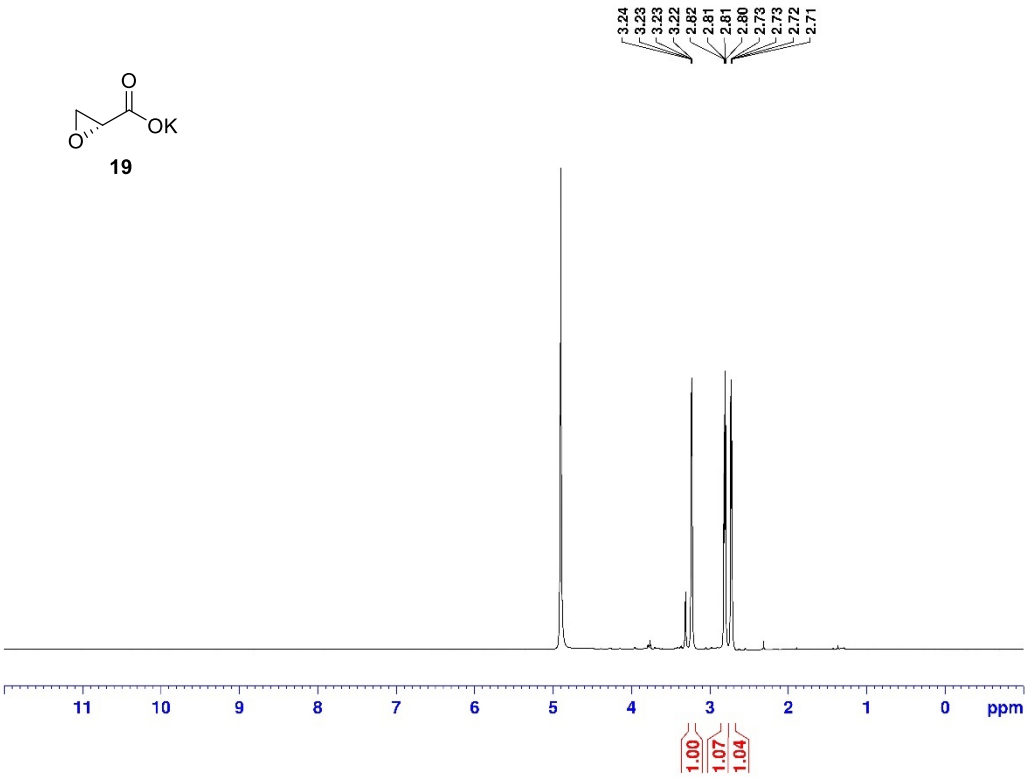
^{13}C-NMR spectrum of compound *rac*-**18** in $CDCl_3$.



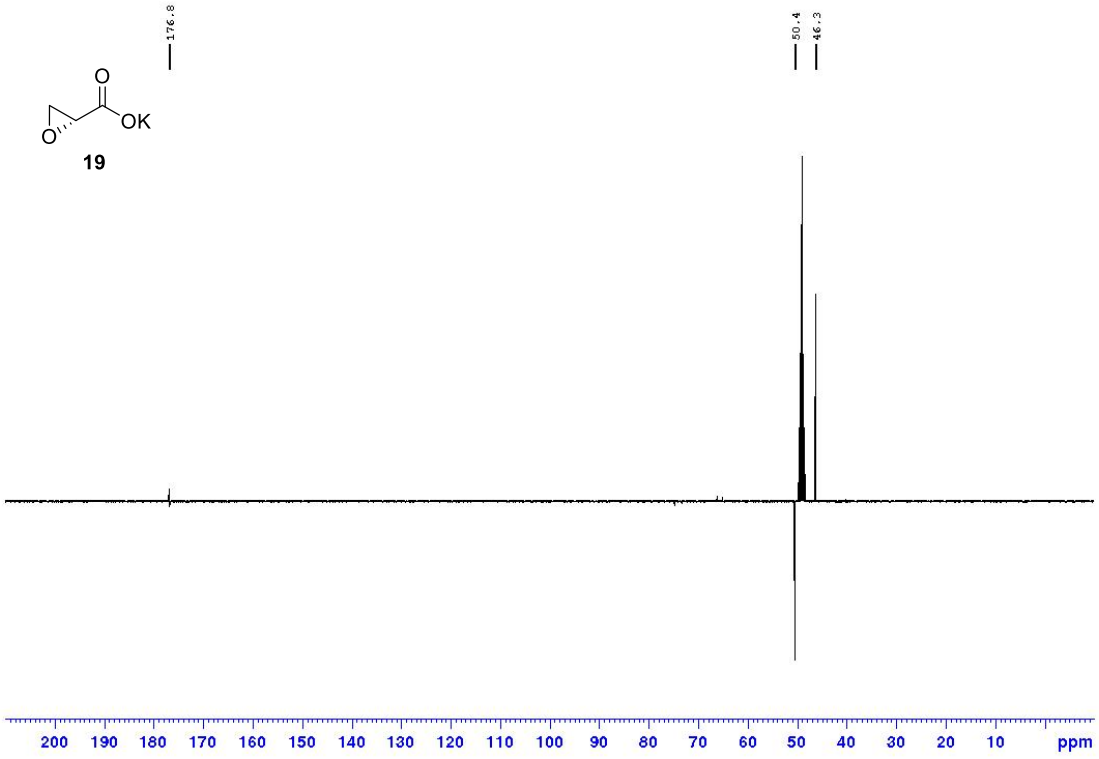
^{1}H-NMR spectrum of compound (*R*)-**18** in $CDCl_3$.



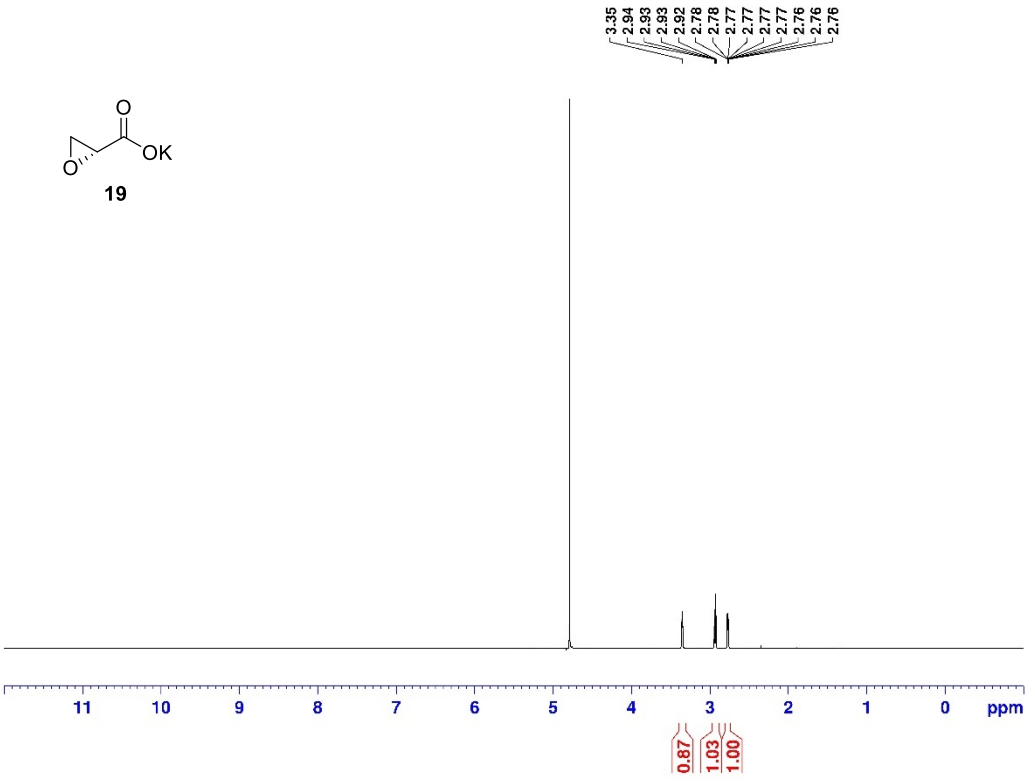
^{13}C-NMR spectrum of compound (*R*)-**18** in $CDCl_3$.



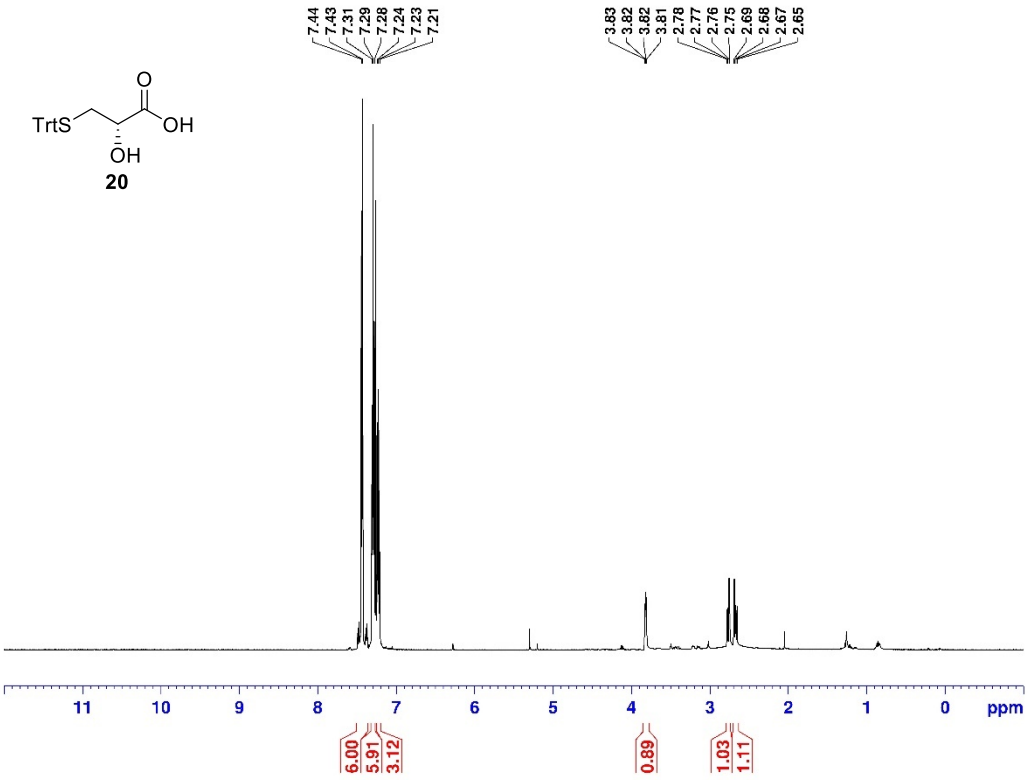
^{1}H-NMR spectrum of compound **19** in CD_3OD.



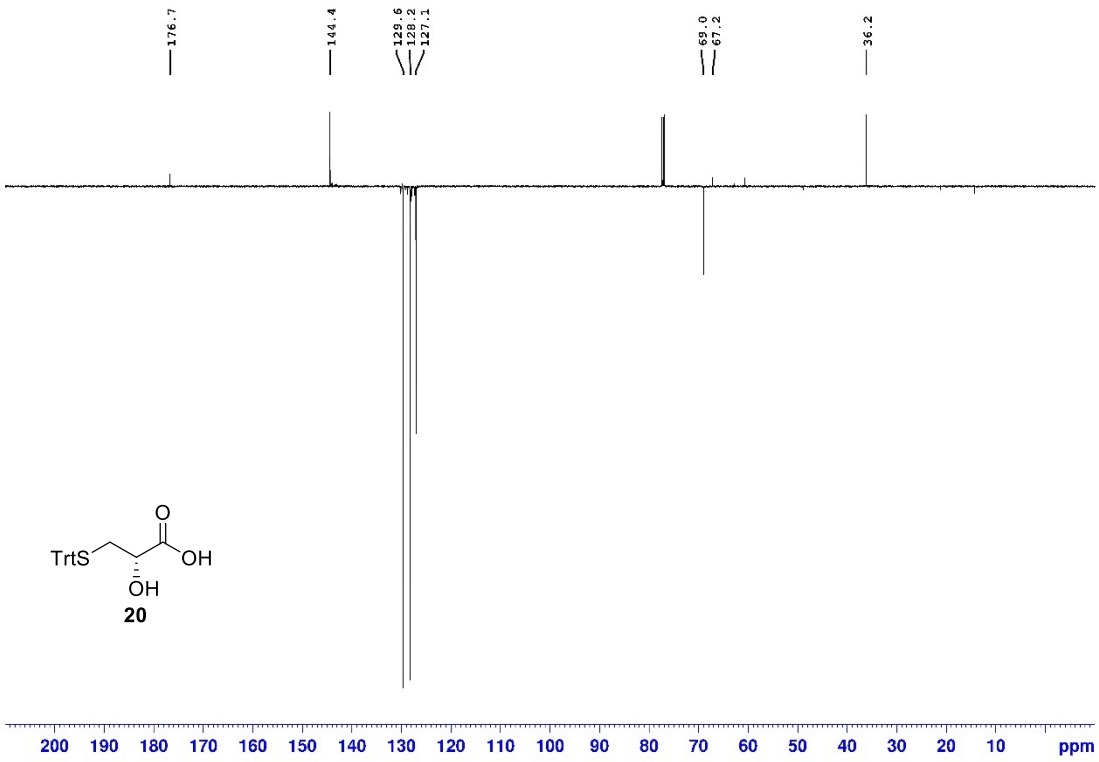
^{13}C-NMR spectrum of compound **19** in CD_3OD.



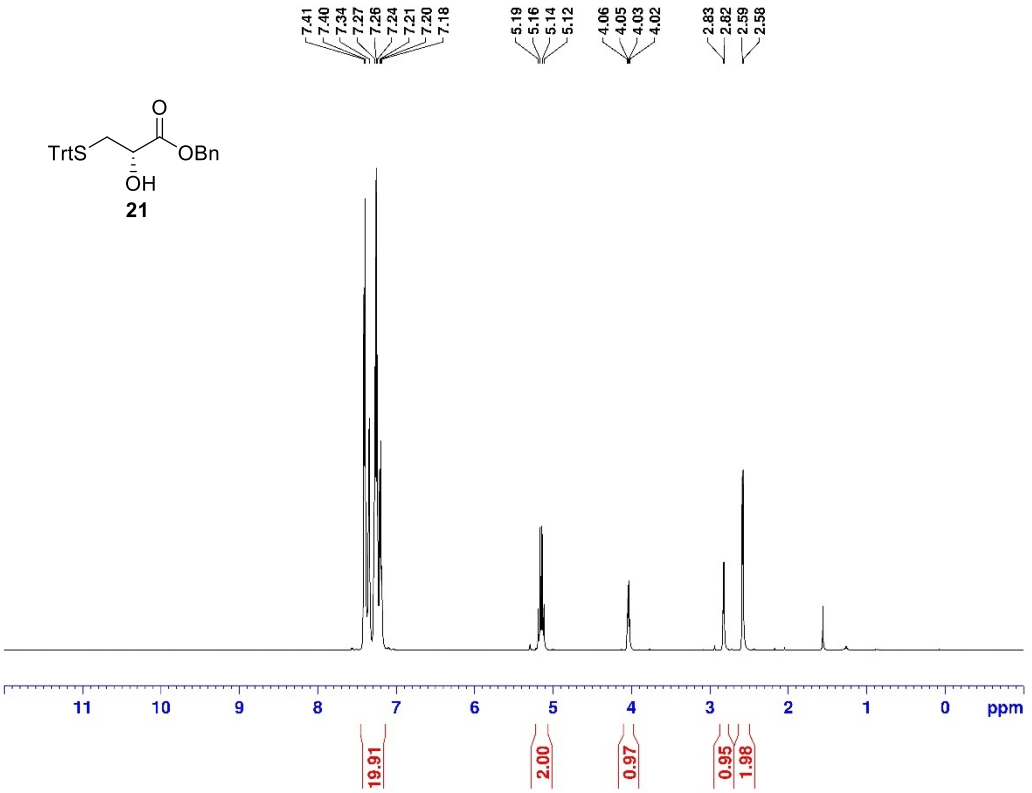
^{1}H-NMR spectrum of compound **19** in D_2O.



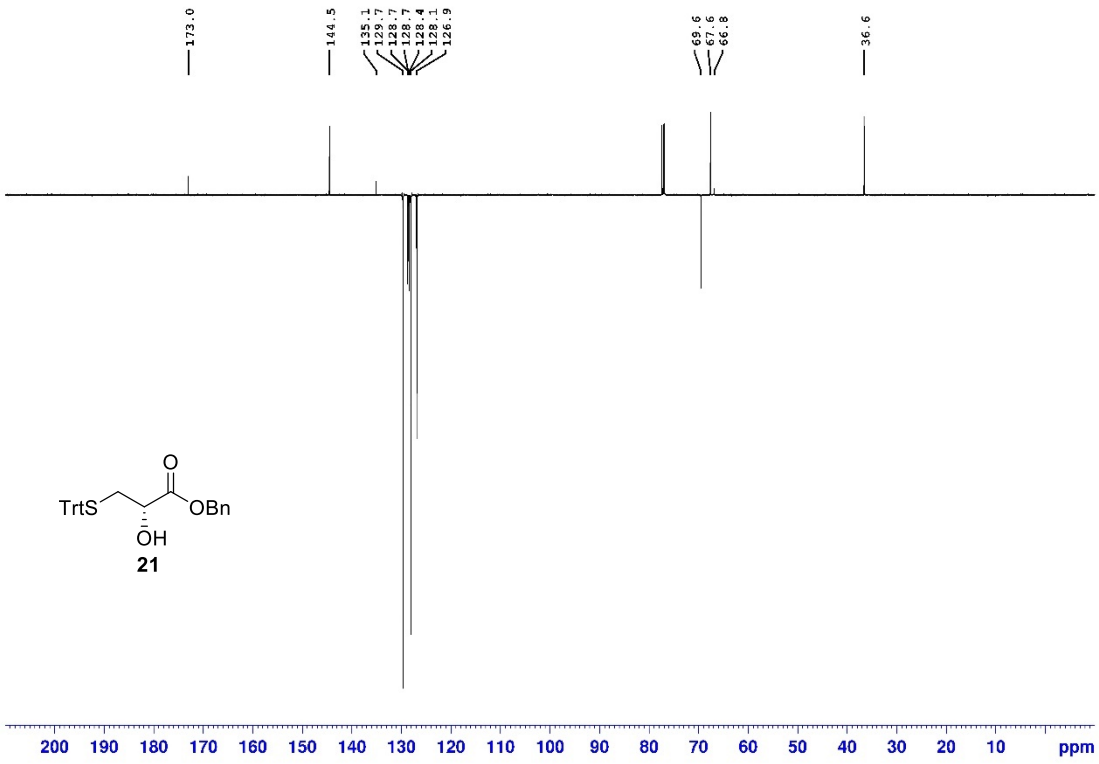
^{1}H-NMR spectrum of compound **20** in $CDCl_3$.



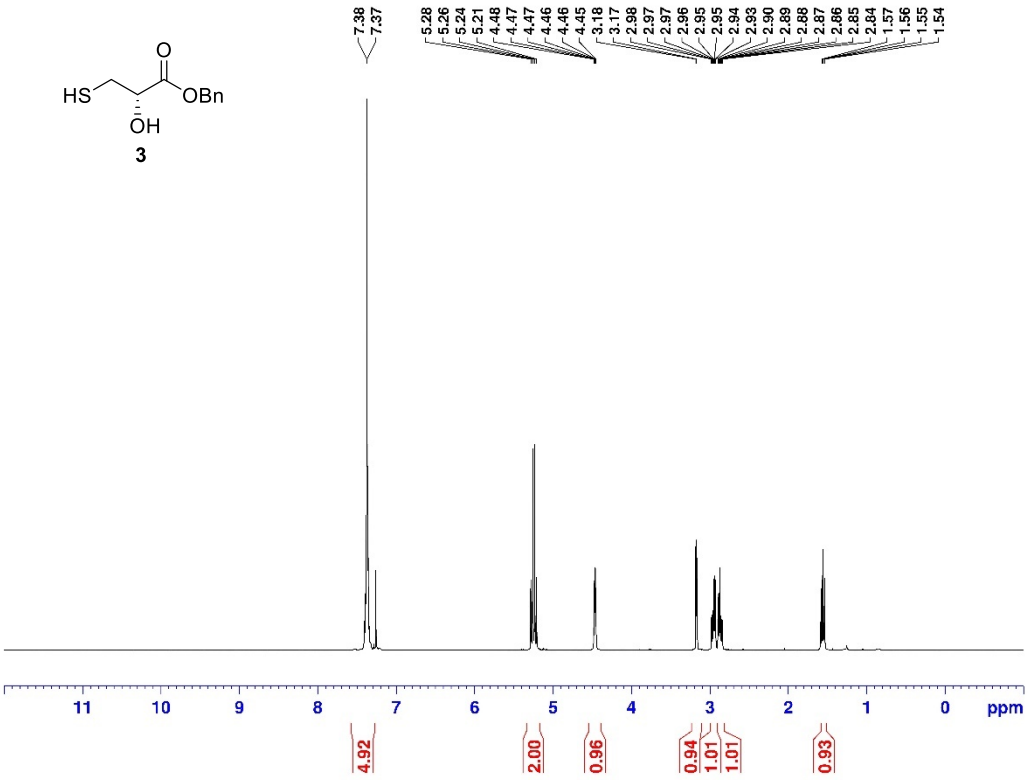
^{13}C-NMR spectrum of compound **20** in $CDCl_3$.



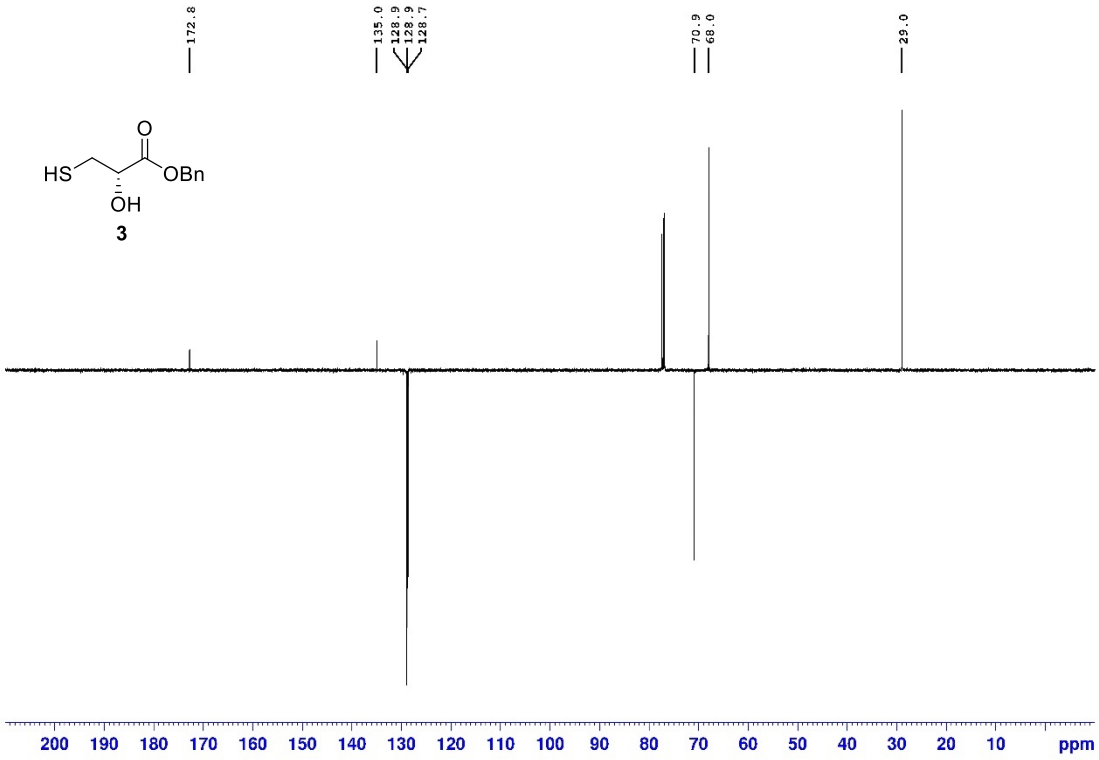
^{1}H-NMR spectrum of compound **21** in $CDCl_3$.



^{13}C-NMR spectrum of compound **21** in $CDCl_3$.



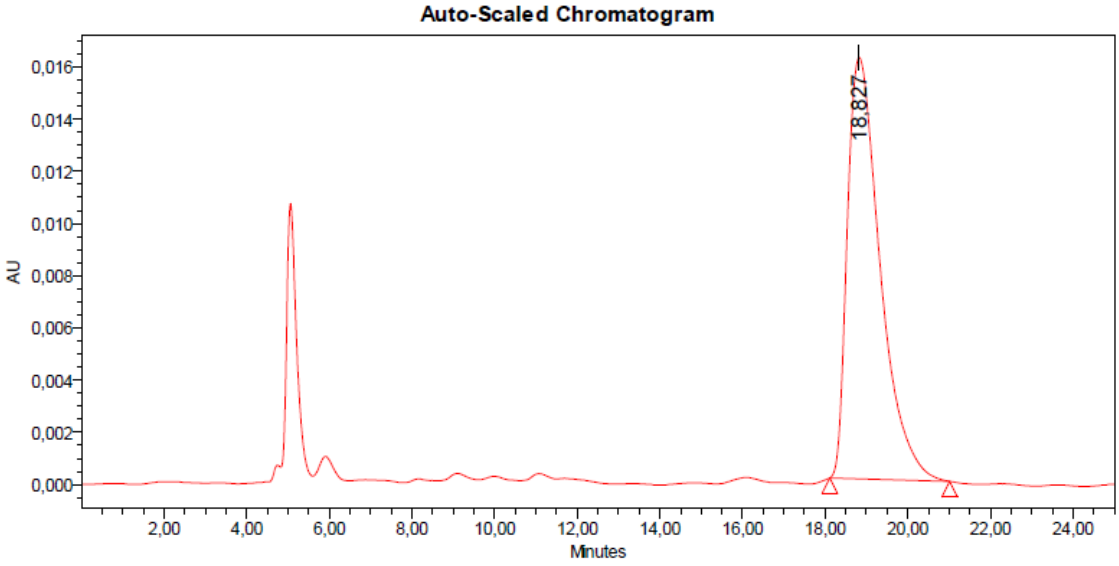
^{1}H-NMR spectrum of compound **3** in $CDCl_3$.



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

SAMPLE INFORMATION

Sample Name:	MSc_230_F1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	MSc
Vial:	4	Acq. Method Set:	ADH H iPr 95 5 Flow 08 215nm
Injection #:	1	Processing Method:	DS AutoProcessing
Injection Volume:	15,00 ul	Channel Name:	2487Channel 1
Run Time:	25,0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	26.05.2020 13:32:57 CEST		
Date Processed:	26.05.2020 14:10:01 CEST		



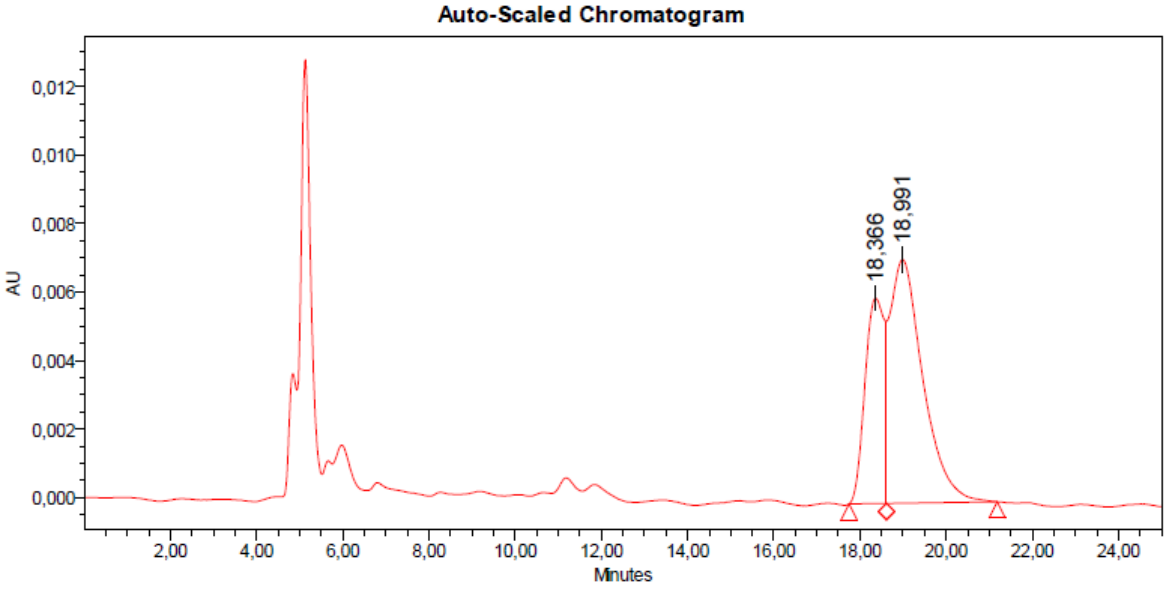
Peak Results

	Name	RT	Area	Height	Amount	Units
1		18,827	889864	16143		

Chromatogram of compound **7** at chiral HPLC.

SAMPLE INFORMATION

Sample Name:	MSc_238_F1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	MSc
Vial:	3	Acq. Method Set:	ADH H iPr 95 5 Flow 08 215nm
Injection #:	1	Processing Method:	DS AutoProcessing
Injection Volume:	15,00 ul	Channel Name:	2487Channel 1
Run Time:	25,0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	26.05.2020 12:56:46 CEST		
Date Processed:	26.05.2020 14:09:25 CEST		



Peak Results

	Name	RT	Area	Height	Amount	Units
1		18,366	187023	5991		
2		18,991	378849	7112		

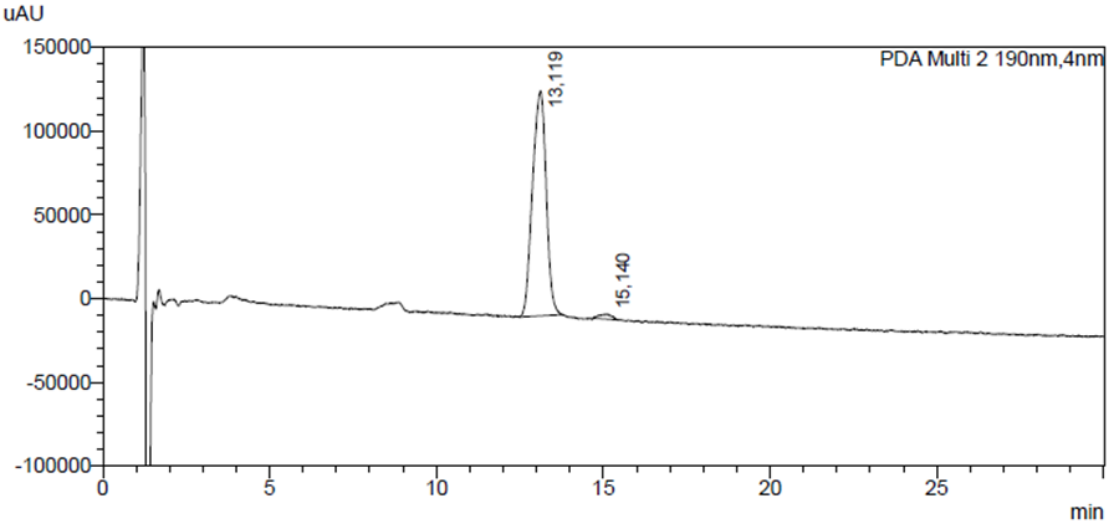
Chromatogram of a mixture of compounds **7**/*epi*-**7** at chiral HPLC.

HPLC-Chromatogram of compound **1** at RP-HPLC

<Sample Information>

Sample Name	: MSc304_F1_40isokrat		
Sample ID	:		
Data Filename	: MSc304_F1_40isokrat_05.11.2020_40_isokrat_30min_001.lcd		
Method Filename	: 40_isokrat_30min.lcm		
Batch Filename	: November2020.lcb		
Vial #	: 1-31	Sample Type	: Unknown
Injection Volume	: 20 uL		
Date Acquired	: 05.11.2020 13:41:55	Acquired by	: System Administrator
Date Processed	: 14.12.2020 10:16:56	Processed by	: System Administrator

<Chromatogram>



<Peak Table>

PDA Ch2 190nm

Peak#	Ret. Time	Area	Height	Area%
1	13,119	3819024	134249	98,007
2	15,140	77650	3040	1,993
Total		3896674	137289	100,000

Minimum inhibitory concentration (MIC) assay. Compound **1** was tested against several bacterial and fungal strains by using a 96-well serial in Mueller-Hinton broth (MHB) media for bacteria and YMG media for filamentous fungi and yeasts as previously described.[1] The selected organisms represent a broad spectrum of pathogens of clinical interest, as well as sensitive indicator strains (Gram-positive bacteria: *Bacillus subtilis*, *Staphylococcus aureus, methicillin-resistant Stapylococcus aureus* [MRSA], *Mycolicibacterium smegmatis*; Gram-negative bacteria: *Acinetobacter baumannii, Chromobacterium violaceum*, *Escherichia coli*, *Pseudomonas aeruginosa*; filamentous fungi: *Mucor hiemalis*; yeasts: *Candida albicans*, *Pichia anomala, Rhodotorula glutinis, Schizosaccharomyces pombe*). Berkeleylactone A (**1**) was dissolved in MeOH (1 mg/mL), diluted to a final range of 66.6 to 0.52 µg/mL and incubated with the test organisms overnight. MeOH was used as negative control. Kanamycin (1.0 mg/mL; 2 µL [*M. smegmatis*]), vancomycin (10 mg/ml; 2 µL [MRSA]), gentamycin (1.0 mg/mL; 2 µL [*P. aeruginosa*]), ciprobay (2.54 mg/ml; 2 µL [*A. baumannii*]), nystatin (1.0 mg/mL; 20 µL [*S. pombe, P. anomala, M. hiemalis, C. albicans, R. glutinis*), and oxytetracycline (1.0 mg/mL; 2 µL [*C. violaceum*, *E. coli*, *S. aureus*] and 20 µL [*B. subtilis*]) were used as positive controls. The lowest inhibitory concentration of compound **1** (where no growth of the test organism was observed) was visually evaluated the next day.

Biofilm inhibition assay. *Staphylococcus aureus* DSM 1104 was taken from −20 °C stock and precultured in 25 mL CASO (casein-peptone soymeal-peptone) medium in a 250 mL flask at 37 °C at 100 rpm for 20 h. The culture solution was adjusted to match the turbidity of a 0.001 McFarland standard OD_{600} and was incubated in 96-well tissue microtiter plates (TPP tissue culture ref.no 92196m Switzerland) in CASO with 4% glucose broth together with the serially diluted compound **1** (10–0.3 µg/mL) and incubated for 18 h at 37 °C. The biofilm inhibition activity of the test compounds was evaluated by crystal violet (CV) staining (Thermo Fisher, Waltham, USA), following previously established protocols.[2,3] In brief, the supernatant was discarded, the biofilm stained with crystal violet for 15 min, washed three times with PBS (phosphate-buffered saline) buffer, the dye in the biofilm was dissolved in 150 µL ethanol (95%), and the absorbance of this extract was finally quantified using a plate reader (Synergy 2, BioTek, Santa Clara, USA) at 530 nm. Standard deviations (SD) of two repeats with duplicate each were 10% or less. Methanol (2.5%) and microporenic acid A (250–2 µg/mL) were used as a negative control and a positive control, respectively.

P. aeruginosa (PA 14) was taken from −20 °C stock and cultured in 25 mL LB medium (Luria-Bertani Broth) in a 250 mL flask at 37 °C at 100 rpm for 18 h. The OD_{600} of the culture solution was measured and adjusted to 0.025 McFarland standard in LB medium. Compound **1** was diluted into 100 µL bacterial solution at the respective concentration (250–2 µg/mL), then the mixture solution was added in 96-well plates in an MBEC Innovatech incubator (MBEC Assay®, Canada). The plates were incubated at 37 °C at 150 rpm for 24 h. The biofilms were established on the pegs under growth conditions. The pegs and plates were rinsed once with PBS buffer, the biofilms on pegs were stained by 150 µL 0.1% CV at room temperature for 15 min and then rinsed twice with PBS buffer. The pegs were transferred into a new plate with 150 µL ethanol (95%) and the absorbance was quantified using a plate reader (Synergy 2, BioTek, Santa Clara, USA) at 550 nm. SD of two repeats with duplicates each were 10% or less. Myxovalargin A and methanol (2.5 %) were used as the positive and negative controls.

Preformed biofilm dispersion assay. *S. aureus* DSM 1104 and *C. albicans* DSM 11225 were taken from −20 °C stock and precultered in 25 mL CASO medium at 37 °C and YPED (Yeast extract Peptone Dextrose) at 30 °C, respectively, at 100 rpm in 250 mL flasks. *S. aureus* was precultured for 20 h, *C. albicans* was cultured for 18 h. The precultured suspensions of *S. aureus* and *C. albicans* were adjusted so that their OD_{600} matched the turbidity of a 0.001 McFarland standard and 0.05 Mc Farland standard, respectively. *S. aureus* was incubated in 96-well tissue plates for 18 h at 150 rpm in 150 mL CASO medium with 4% glucose broth. For *C. albicans*, the 150 µL fungal solution was added to 96-well non-tissue microtiter plates (Falcon non-tissue plate ref.no 351172) for 90 min at 37 °C at 150 rpm. The supernatant was removed from the wells and 150 µL of the respective media (fresh) was added to the wells, together with the serially diluted compound **1** (*S. aureus*: 250–2 µg/mL; *C. albicans*: 250–2 µg/mL). Due to strong activities in the *C. albicans* assay, a repetition with compound **1** at a higher dilution was carried out (*C. albicans*: 10-0.3 µl/ml). The plates were incubated for a further 24 h at 37 °C. Staining of the preformed biofilm, and the control runs were carried out as described above. SD of two repeats with duplicates each were 10% or less. Methanol (2.5%) and microporenic acid A (250–2 µg/mL) were used as negative and a positive controls.

Cytotoxicity assay. The evaluation of *in vitro* cytotoxicity (IC_{50}) was performed with mouse fibroblast cell line L929 and mammalian HeLa KB3.1 cancer cells for compound **1** as previously described.[1] The compound was dissolved in MeOH (1 mg/mL), MeOH itself was

used as negative control, and epothilone B (1 mg/mL) was used as a positive control. After incubating the cell lines with the serially diluted test compound **1** (37–0.6 x 10^{-3} µg/mL) for five days, the cells were dyed using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), which is only converted to its purple formazan derivative by living cells. The absorption at 595 nm was measured using a microplate reader, and the percentage of cell viability was calculated. The half maximum inhibitory concentration was calculated and expressed as IC_{50} (µM).

Literature

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