Supporting Information

Modular and scalable synthesis of nematode pheromone ascarosides: implications in eliciting plant defense

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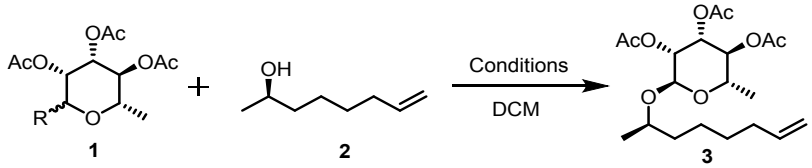
List of Supporting Information

Table S1. Optimization on glycosylation of **1** and **2**



Entry	R	Lewis acids	Temp. (°C)	Conv. (%)	Yield (%)[a]
1	**OAc**	TMSOTf	-40 °C-RT	100	25
2	**OAc**	$BF_3 \cdot Et_2O$	-40 °C-RT	N.R.	-
3	**OTCA**	TMSOTf	-40 °C-RT	100	32
4	**OTCA**	$BF_3 \cdot Et_2O$	-40 °C-RT	100	31
5	**OTCA**	$BF_3 \cdot Et_2O$	-15 °C-RT	100	29
6	**OTCA**	**$BF_3 \cdot Et_2O$**	**-78 °C-RT**	**100**	**33**
7	**STol**	TfOH	-40 °C-RT	N.R.	-
8	**STol**	$Cu(OTf)_2$	-78 °C-RT	N.R.	-
9	**STol**	TMSOTf	-40 °C-RT	100	21
10	**STol**	AgOTf	-40 °C-RT	100	0
11	**STol**	Tf_2O	-40 °C-RT	80	complex

[a] The isolated yield of the desired product.

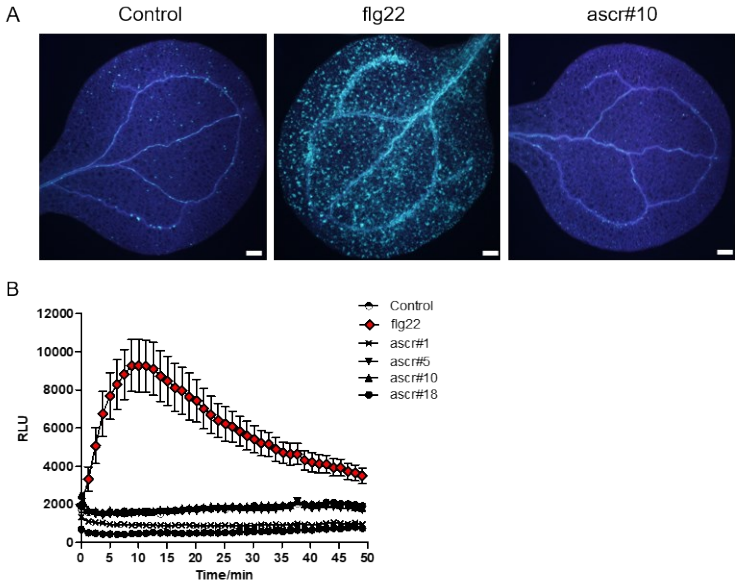


Figure S1. (A) Ascaroside treatment induces callose deposition in *Arabidopsis thaliana* Col-0. 10-Day-old seedings were treated with 1 μM flg22 or 1 μM ascr#10 for 24 h, the treatment with H_2O was as control and callose was detected by aniline blue staining. (B) Ascaroside treatment induces ROS burst in *Arabidopsis thaliana* Col-0. 10 day-old seedings were treated with 50 nM flg22 or 1 μM ascarosides（ascr#1, ascr#5, ascr#10, ascr#18）and the relative light units (RLU) were recorded. Values represent the mean ± SE (n = 8).

Table S2. Primers used for qRT-PCR

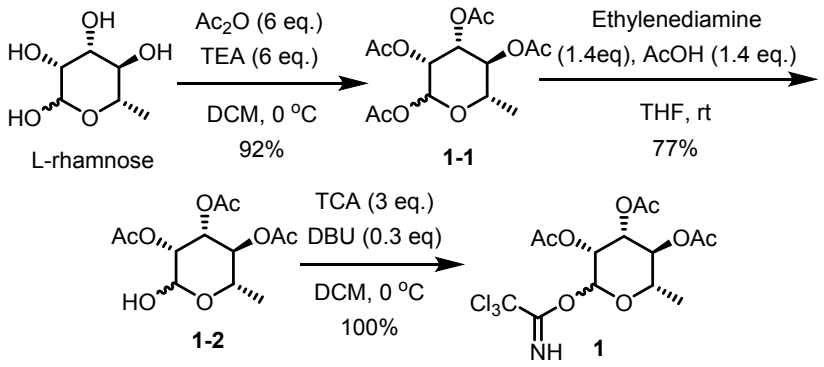
Name	Sequence（5’-3’）
qAtUBQ-fw	GGCCTTGTATAATCCCTGATGAATAAG
qAtUBQ-rv	AAAGAGATAACAGGAACGGAAACATAG
AtAOS-fw	TCTTCTCTTCGCCACGTGC
AtAOS-rv	GGTTATGAACTTGATGACCCGC
AtPDF1.2-fw	TCATGGCTAAGTTTGCTTCC
AtPDF1.2-rv	AATACACACGATTAGCACC
AtLOX2-fw	TTGCTCGCCAGACACTTGC
AtLOX2-rv	GGGATCACCATAAACGGCC
AtPR1-fw	TCGTCTTTGTAGCTCTTGTAGGTG
AtPR1-rv	TAGATTCTCGTAATCTCAGCTCT

General experimental information.

All reactions were performed using flame-dried glassware unless otherwise noted. All reagents were commercially available and used without further purification unless indicated otherwise. CH_3CN, DCM, DMF, DCE were distilled over CaH_2. THF and ether were distilled over Na under argon. Thin layer chromatography was carried out on silica gel GF254 plates (0.25 mm layer thickness). Flash chromatography was performed with 300–400 mesh silica gels. Visualization of the developed chromatogram was performed by fluorescence quenching or by ceric ammonium molybdate, or $KMnO_4$ stain. Reported yields are for isolated, spectroscopically pure compounds.

^{1}H and ^{13}C NMR experiments were performed on a Bruker AM-400 and DRX-500 NMR spectrometer at ambient temperature. The residual solvent protons (^{1}H) or the solvent carbons (^{13}C) were used as internal standards. ^{1}H NMR data are presented as follows: chemical shift in ppm (multiplicity, coupling constant, integral). The following abbreviations are used in reporting NMR data: s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; qt, quartet of triplets; dd, doublet of doublets; dt, doublet of triplets; m, multiplet. HRMS spectra were obtained on an Thermo LC-LTQ orbitrap Velos Pro ETD.

Experimental procedures.



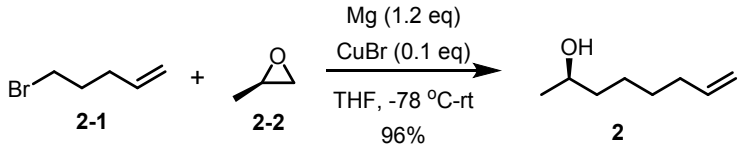
(3R,4R,5S,6S)-2-hydroxy-6-methyltetrahydro-2H-pyran-3,4,5-triyl triacetate (1). [1]

Step 1: To a mixture of L-rhamnose monohydrate (9.1 g, 50 mmol) and Et_3N (41.6 mL, 300 mmol) in DCM (80 mL) was added Ac_2O (28.3 mL, 300 mmol) dropwise at 0 °C. Upon completion of the addition, the mixture was allowed to warm to room temperature and was stirred for another 14 h until no starting material was detected by TLC. The mixture was washed with H_2O (100 mL × 3), saturated aqueous $NaHCO_3$ solution (100 mL × 3) and brine (100 mL × 3) successively. The organic layers were dried over anhydrous $NaSO_4$, filtered, and concentrated in vacuum. The crude product was purified by silica gel column chromatography with DCM to afford **1-1** (15.3 g, 46.1 mmol, 92%) as brown oil.

Step 2: To a solution of ethylene diamine (1.68 mL, 25.2 mmol) in THF (32 mL) was added AcOH (1.44 mL, 25.2 mmol), which was allowed to stir at room temperature for 0.5 h. Then a solution of **1-1** (6.0 g, 18 mmol) in THF (8 mL) was added to the mixture and was stirred at room temperature for another 10 h. The solvent was then removed in vacuum and the residue was diluted with ethyl acetate (30 mL). The mixture was washed with H_2O (20 mL × 3), saturated aqueous $NaHCO_3$ solution (20 mL × 3) and brine (20 mL × 3). The organic layers were dried over anhydrous $NaSO_4$, filtered, and concentrated in vacuum. The crude product was purified by silica gel column chromatography (petrol ether : ethyl acetate = 2 : 1) to afford **1-2** (4.0 g, 13.8 mmol, 77%) as yellow solid.

Step 3: To a solution of **1-2** (1.7 g, 6.0 mmol) in DCM (21 mL) at 0 °C were added DBU (0.27 mL, 1.8 mmol) and Cl_3CCN (1.8 mL, 18 mmol) successively. The resulting mixture was allowed to stir at room temperature for 2 h until no starting

material was detected by TLC. The solvent was then removed in vacuum and the residue was purified by silica gel column chromatography (petrol ether : ethyl acetate = 4 : 1) to afford **1** (2.6 g, 6.0 mmol, 100%) as brown powder.

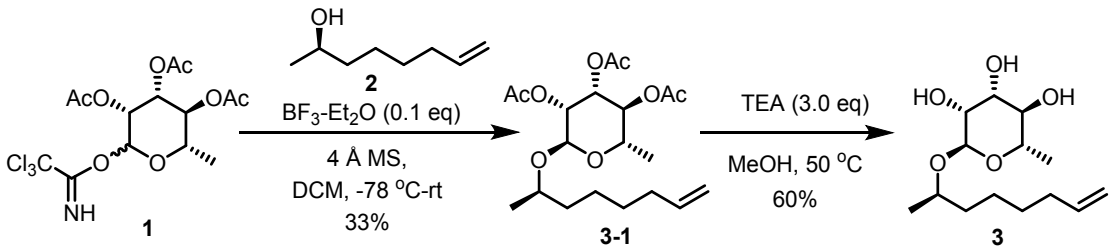


Procedure for the synthesis of (R)-(-)-7-Octen-2-ol (2).[2]

To a suspension of Mg (1.7 g, 70.5 mmol) and catalytic amount of cuprous bromide in dry THF (9 mL) was added a solution of 5-bromo-1-pentene **2-1** (8.3 mL, 70.5 mmol) in dry THF (60 mL) dropwise over 30 min. The resulting mixture was stirred at room temperature for 3 h until all the Mg was dissolved. Subsequently, to the suspension of (*R*)-(+)-1,2-epoxypropane **2-2** (4.0 mL, 57.0 mmol) and CuBr (816 mg, 5.7 mmol) in dry THF (69 mL) was added a freshly prepared 4-pentenylmagnesium bromide in THF (69 mL, 70.5 mmol) dropwise at -78 °C, which was allowed to slowly warm to room temperature and was stirred at this temperature for another 4 h until no starting material was detected by TLC. The reaction was quenched with saturated aqueous NH_4Cl solution (50 mL) and was extracted with Et_2O (100 mL × 3). The organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuum. The crude product was purified by silica gel column chromatography (*n*-pentane : Et_2O = 4 : 1) to afford **2** (7.0 g, 54.7 mmol, 96%) as colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 5.72 (dq, *J* = 11.7, 7.0 Hz, 1H), 4.92 (d, *J* = 17.2 Hz, 1H), 4.86 (d, *J* = 10.2 Hz, 1H), 3.72 – 3.63 (m, 1H), 1.98 (d, *J* = 6.3 Hz, 2H), 1.45 – 1.32 (m, 4H), 1.27 – 1.19 (m, 2H), 1.09 (d, *J* = 6.4 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 138.8, 114.2, 67.7, 39.0, 33.7, 28.9, 25.2, 23.2.



(2S,3R,4R,5R,6R)-2-methyl-6-(((R)-oct-7-en-2-yl)oxy)tetrahydro-2H-pyran-3,4,5-triol (3)

Step 1: To a suspension of **1** (867 mg, 2 mmol), **2** (256 mg, 2 mmol), and 4Å molecular sieves in dry DCM (8 mL) under N_2 at -78 ℃ was added $BF_3 \cdot Et_2O$ (25.3 μL, 0.2 mmol) dropwise. The mixture was allowed to warm to room temperature slowly and was stirred for another 1 h until no starting material was detected by TLC. The reaction was quenched with saturated aqueous $NaHCO_3$ solution (10 mL), and was extracted with DCM (10 mL × 3). The organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuum. The crude product was purified by silica gel column chromatography (petrol ether : ethyl acetate = 4 : 1) to afford the coupling products as a 1:1 mixture of the diastereoisomers. The desired product **3-1** was isolated as yellow oil (264 mg, 0.66 mmol, 33%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.79 (ddt, *J* = 16.9, 10.2, 6.7 Hz, 1H), 5.27 (dd, *J* = 10.1, 3.5 Hz, 1H), 5.15 (dd, *J* = 3.4, 1.7 Hz, 1H), 5.04 (t, *J* = 10.0 Hz, 1H), 4.98 (dd, *J* = 17.1, 1.7 Hz, 1H), 4.92 (dd, *J* = 10.2, 0.8 Hz, 1H), 4.81 (d, *J* = 1.4 Hz, 1H), 3.90 (dq, *J* = 9.9, 6.3 Hz, 1H), 3.77 – 3.67 (m, 1H), 2.13 (s, 3H), 2.06 – 2.02 (m, 5H), 1.97 (s, 3H), 1.48 – 1.34 (m, 6H), 1.18 (d, *J* = 6.3 Hz, 3H), 1.11 (d, *J* = 6.1 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.2, 170.0, 169.9, 138.7, 114.4, 94.9, 72.9, 71.2, 70.6, 69.2, 66.4, 36.7, 33.7, 28.7, 25.1, 21.0, 20.8, 20.7, 18.9, 17.3.

HR-ESI-MS (m/z) calcd for $C_{20}H_{32}NaO_8^+$ $[M+Na]^+$ 423.1989, found 423.1994.

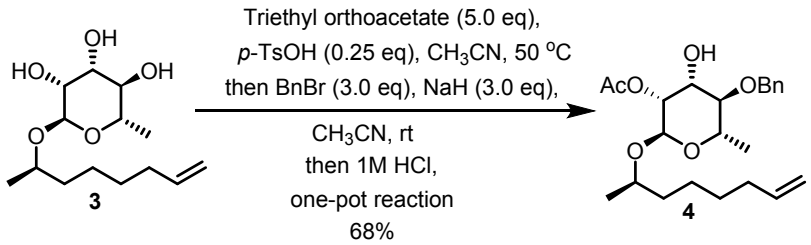
Step 2: To a solution of **3-1** (100 mg, 0.25 mmol) in MeOH (40 mL) was added Et_3N (0.1 mL, 0.75 mmol) dropwise. The mixture was allowed to stir at 50 °C for 3 h until no starting material was detected by TLC. The mixture was concentrated in vacuum and purified by silica gel column chromatography (petrol ether : ethyl acetate = 1 : 10) to afford **3** (40 mg, 0.15 mmol, 60%) as yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.87 – 5.74 (m, 1H), 5.01 (d, *J* = 17.1 Hz, 1H), 4.95 (d, *J* = 10.2 Hz, 1H), 4.88 (s, 1H), 3.87 (s, 1H), 3.82 – 3.64 (m, 3H), 3.49 (t, *J* = 9.2 Hz, 1H), 2.06 (d, *J* = 5.9 Hz, 2H), 1.62 – 1.49 (m, 1H), 1.47 – 1.34 (m, 5H), 1.30 (d, *J* = 5.1 Hz, 3H), 1.12 (d, *J* = 4.7 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 138.8, 114.5, 97.1, 72.7, 72.1, 71.8, 71.7, 68.4, 36.9,

33.7, 28.8, 25.2, 18.9, 17.5.

HR-ESI-MS (m/z) calcd for $C_{14}H_{26}NaO_5^+$ $[M+Na]^+$ 297.1672, found 297.1676.



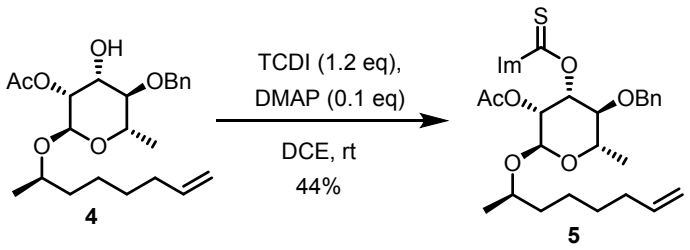
(2R,3R,4R,5R,6S)-5-(benzyloxy)-4-hydroxy-6-methyl-2-(((R)-oct-7-en-2-yl)oxy) tetrahydro-2H- pyran-3-yl acetate (4).

To a solution of **3** (208 mg, 0.76 mmol) in dry CH_3CN (8 mL) at 50 °C were added triethylorthoacetate (691 μL, 3.8 mmol) and *p*-TsOH (39.3 mg, 0.2 mmol) successively. The mixture was allowed to stir at 50 °C for 8 h until no starting material was detected by TLC. Then Et_3N (27 μL, 0.2 mmol) was added to neutralize the solution. Then NaH (54.7 mg, 2.3 mmol) and BnBr (278 μL, 2.3 mmol) were added successively. The mixture was allowed to stir at room temperature until all the intermediate was completely consumed. MeOH (2 mL) was carefully added to quench excess NaH. The mixture was diluted with DCM (20 mL). The organic layer was successively neutralized with 1.0 mol/L aqueous HCl solution (20 mL × 3), washed with saturated aqueous $NaHCO_3$ solution (20 mL × 3) and water (20 mL × 3), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuum. The crude product was purified by silica gel column chromatography (petrol ether : ethyl acetate = 4 : 1) to afford **4** (210 mg, 0.52 mmol, 68%) as yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.30 (m, 5H), 5.82 (ddt, *J* = 16.9, 10.2, 6.7 Hz, 1H), 5.05 (dt, *J* = 3.0, 1.5 Hz, 1H), 5.01 (ddd, *J* = 17.1, 3.6, 1.6 Hz, 1H), 4.96 (ddt, *J* = 10.2, 2.2, 1.2 Hz, 1H), 4.87 (d, *J* = 6.5 Hz, 1H), 4.85 (d, *J* = 3.0 Hz, 1H), 4.73 (d, *J* = 11.1 Hz, 1H), 4.12 (dd, *J* = 9.4, 3.6 Hz, 1H), 3.86 – 3.79 (m, 1H), 3.78 – 3.71 (m, 1H), 3.38 (t, *J* = 9.5 Hz, 1H), 2.20 – 2.16 (m, 3H), 2.11 – 2.02 (m, 2H), 1.61 – 1.50 (m, 1H), 1.48 – 1.37 (m, 5H), 1.36 (d, *J* = 6.3 Hz, 3H), 1.13 (d, *J* = 6.1 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 171.0, 138.9, 138.3, 128.5, 128.0, 127.9, 114.4, 94.8, 81.8, 75.2, 73.6, 72.5, 70.2, 67.6, 36.9, 33.7, 28.8, 25.1, 21.2, 18.9, 18.0.

HR-ESI-MS (m/z) calcd for $C_{23}H_{34}NaO_6^+$ $[M+Na]^+$ 429.2248, found 429.2249.



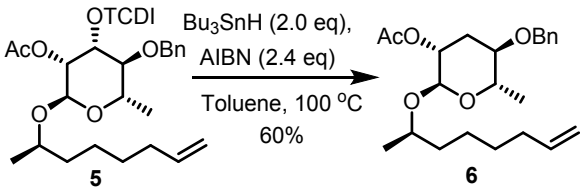
(2R,3R,4R,5S,6S)-4-((1H-imidazole-1-carbonothioyl)oxy)-5-(benzyloxy)-6-methyl-2-(((R)-oct-7-en-2-yl)oxy) tetrahydro-2H-pyran-3-yl acetate (5).

To a solution of **4** (456 mg, 1.1 mmol) and TCDI (231 mg, 1.3 mmol) in DCE (2 mL) was added DMAP (13.4 mg, 0.11 mmol) in one portion. The mixture was stirred at room temperature for 5 h until all the starting material was consumed. The solvent was then removed in vacuum and the residue was purified by silica gel column chromatography (petrol ether : ethyl acetate = 4 : 1) to afford **5** (249 mg, 0.48 mmol, 44%) as yellow powder.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H), 7.53 – 7.48 (m, 1H), 7.27 – 7.16 (m, 5H), 7.01 (d, J = 0.7 Hz, 1H), 6.09 (dd, J = 9.5, 3.4 Hz, 1H), 5.81 (ddt, J = 16.9, 10.2, 6.7 Hz, 1H), 5.33 (dd, J = 3.3, 1.9 Hz, 1H), 5.04 – 4.92 (m, 2H), 4.89 (d, J = 1.5 Hz, 1H), 4.68 (d, J = 11.3 Hz, 1H), 4.57 (d, J = 11.3 Hz, 1H), 3.99 (dq, J = 9.3, 6.2 Hz, 1H), 3.75 (dt, J = 12.8, 7.8 Hz, 2H), 2.11 (s, 3H), 2.09 – 2.03 (m, 2H), 1.65 – 1.54 (m, 1H), 1.51 – 1.40 (m, 5H), 1.39 (s, 3H), 1.15 (d, J = 6.1 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 182.5, 169.8, 138.8, 137.3, 136.4, 130.9, 128.4, 128.1, 127.7, 118.0, 114.5, 94.7, 80.1, 79.1, 75.3, 73.0, 70.4, 68.0, 36.7, 33.7, 28.8, 25.0, 20.9, 18.8, 18.0.

HR-ESI-MS (m/z) calcd for $C_{27}H_{36}N_2NaO_6S^+$ $[M+Na]^+$ 539.2186, found 539.2189.



(2R,3R,5R,6S)-5-(benzyloxy)-6-methyl-2-(((R)-oct-7-en-2-yl)oxy)tetrahydro-2H-

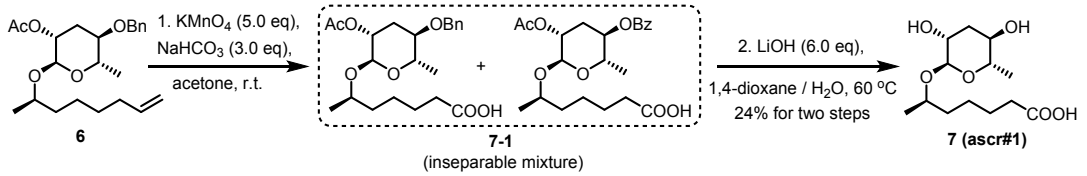
pyran-3-yl acetate (6).

To a solution of Bu_3SnH (226 μL, 0.84 mmol) in toluene (4 mL) at 100 °C under N_2 were added AIBN (164.2 mg, 1.0 mmol) and a solution of **5** (214 mg, 0.42 mmol) in toluene (2 mL) successively. The resulting mixture was stirred at 100 °C for 2 h until no starting material was detected by TLC. The solvent was then removed in vacuum and the residue was purified by silica gel column chromatography (petrol ether : ethyl acetate = 15 : 1) to afford **6** (98 mg, 0.25 mmol, 60%) as yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.26 (m, 5H), 5.80 (ddt, *J* = 16.9, 10.2, 6.7 Hz, 1H), 4.99 (dd, *J* = 17.1, 1.7 Hz, 1H), 4.93 (dd, *J* = 10.2, 0.9 Hz, 1H), 4.85 (d, *J* = 0.9 Hz, 1H), 4.71 (s, 1H), 4.61 (d, *J* = 11.5 Hz, 1H), 4.47 (d, *J* = 11.5 Hz, 1H), 3.88 – 3.80 (m, 1H), 3.80 – 3.71 (m, 1H), 3.39 – 3.31 (m, 1H), 2.20 (dt, *J* = 13.5, 3.5 Hz, 1H), 2.07 (s, 3H), 2.06 – 2.01 (m, 2H), 1.94 – 1.86 (m, 1H), 1.59 – 1.50 (m, 1H), 1.46 – 1.34 (m, 5H), 1.28 (d, *J* = 6.2 Hz, 3H), 1.11 (d, *J* = 6.1 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.3, 138.9, 138.3, 128.4, 127.8, 127.7, 114.4, 93.6, 75.2, 72.0, 71.3, 71.2, 68.2, 37.0, 33.7, 29.4, 28.9, 25.1, 21.2, 19.0, 18.0.

HR-ESI-MS (m/z) calcd for $C_{23}H_{34}NaO_5^+$ $[M+Na]^+$ 413.2298, found 413.2300.



6R-(3'R,5'R-dihydroxy-6'S-methyl-(2H)-tetrahydropyran-2'-yloxy) heptanoic acid (7, ascr#1).

Step 1: To a solution of **6** (47.7 mg, 0.12 mmol) in acetone (5 mL) were added $NaHCO_3$ (30.8 mg, 0.37 mmol) and $KMnO_4$ (96.6 mg, 0.61 mmol) successively. The resulting mixture was stirred at room temperature for 12 h until no starting material was detected by TLC. The aqueous layer was acidified to *p*H 2 with aqueous HCl solution (1.0 mol/L) and extracted with ethyl acetate (10 mL × 2). The organic layers were washed with brine (20 mL × 2), dried over anhydrous Na_2SO_4, and concentrated in vacuum to afford an inseparable mixture of **7-1**, which was used for the next step without further purification .

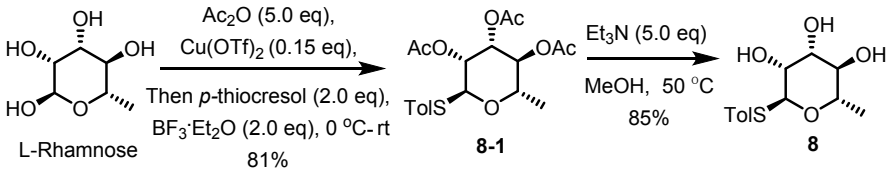
Step 2: To a solution of **7-1** from the previous step in 1,4-dioxane (4.7 mL) was added aqueous LiOH (17.6 mg, 0.74 mmol in 0.6 mL H_2O). The resulting mixture was stirred at 60 °C for 6 h until the starting material was fully consumed. The aqueous layer was acidified to *p*H 2 with aqueous HCl solution (1.0 mol/L), then extracted with Et_2O (10 mL × 3). The organic layers were washed with brine (10 mL × 3), dried over anhydrous Na_2SO_4, and concentrated in vacuum. The crude product was purified by silica gel column chromatography (DCM : MeOH = 15 : 1) to afford **7** (8 mg, 0.029 mmol, 24% for two steps) as colorless oil.

The spectral data of **7** are identical to those of a previous report.[2]

^{1}H NMR (400 MHz, CD_3OD) δ 4.66 (s, 1H), 3.85 – 3.76 (m, 1H), 3.74 (d, *J* = 0.9 Hz, 1H), 3.67 – 3.59 (m, 1H), 3.57 – 3.47 (m, 1H), 2.32 (t, *J* = 7.4 Hz, 2H), 1.97 (dt, *J* = 12.9, 3.6 Hz, 1H), 1.83 – 1.73 (m, 1H), 1.70 – 1.56 (m, 3H), 1.55 – 1.39 (m, 3H), 1.23 (d, *J* = 6.2 Hz, 3H), 1.14 (d, *J* = 6.1 Hz, 3H).

^{13}C NMR (101 MHz, CD_3OD) δ 177.6, 97.4, 72.2, 71.1, 69.9, 68.3, 38.0, 35.9, 34.8, 26.3, 26.0, 19.3, 18.0.

HR-ESI-MS (m/z) calcd for $C_{13}H_{24}NaO_6^+$ $[M+Na]^+$ 299.1465, found 299.1464.



(2S,3S,4R,5R,6S)-2-methyl-6-(p-tolylthio)tetrahydro-2H-pyranol (8)[3]

Step 1: To a mixture of L-rhamnose monohydrate (3.6 g, 20 mmol) and acetic anhydride (9.4 mL, 100 mmol) was added $Cu(OTf)_2$ (11.5 mg, 0.03 mmol) at 0 °C under N_2. Then the ice bath was removed, and the mixture was stirred at room temperature for 6 h. When reaction was completed according to TLC, *p*-thiocresol (4.8 mL, 40 mmol) and $BF_3 \cdot Et_2O$ (5.0 mL, 40 mmol) were sequentially added. The mixture was then allowed to warm to room temperature and was stirred for another 2 h until all the starting material was consumed by TLC. The reaction was quenched with saturated aqueous $NaHCO_3$ solution (50 mL), and the resulting mixture was extracted with ethyl acetate (80 mL × 3). The organic layers were washed with brine

(100 mL × 3), dried over anhydrous $NaSO_4$, filtered, and concentrated in vacuum. The crude product was purified by silica gel column chromatography (petrol ether : ethyl acetate = 4 : 1) to afford **8-1** (6.4 g, 16.2 mmol, 81%) as white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (d, *J* = 8.1 Hz, 2H), 7.16 – 7.11 (m, 2H), 5.50 (dd, *J* = 3.3, 1.6 Hz, 1H), 5.34 (s, 1H), 5.30 (dd, *J* = 10.1, 3.4 Hz, 1H), 5.15 (t, *J* = 9.9 Hz, 1H), 4.38 (dq, *J* = 9.7, 6.2 Hz, 1H), 2.33 (s, 3H), 2.14 (s, 3H), 2.09 (s, 3H), 2.02 (s, 3H), 1.25 (d, *J* = 6.3 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.0, 170.0, 169.9, 138.2, 132.5, 130.0, 129.4, 86.0, 71.3, 71.2, 69.4, 67.7, 21.1, 20.9, 20.8, 20.7, 17.3.

HR-ESI-MS (m/z) calcd for $C_{19}H_{24}NaO_7S^+$ $[M+Na]^+$ 419.1135, found 419.1131.

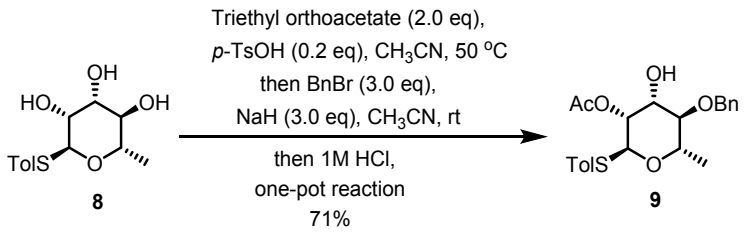
Step 2: To a solution of **8-1** (3.4 g, 8.6 mmol) in MeOH (40 mL) was added Et_3N (5.9 mL, 43.0 mmol). The mixture was stirred at 50 °C overnight until no starting material was detected by TLC. The solvent was removed in vacuum. The residue was subjected to silica gel column chromatography (petrol ether : ethyl acetate = 1 : 10) to afford **8** (1.97 g, 7.3 mmol, 85%) as white solid.

The spectral data of **8** are identical to those of a previous report.[3]

^{1}H NMR (400 MHz, CD_3OD) δ 7.37 (d, *J* = 8.0 Hz, 2H), 7.15 (d, *J* = 7.0 Hz, 2H), 5.32 (s, 1H), 4.15 – 4.02 (m, 2H), 3.71 – 3.62 (m, 1H), 3.48 (t, *J* = 9.4 Hz, 1H), 2.33 (s, 3H), 1.29 (d, *J* = 6.2 Hz, 3H).

^{13}C NMR (101 MHz, CD_3OD) δ 137.4, 131.9, 130.8, 129.4, 89.1, 72.8, 72.4, 71.5, 69.5, 19.7, 16.5.

HR-ESI-MS (m/z) calcd for $C_{13}H_{18}NaO_4S^+$ $[M+Na]^+$ 293.0818, found 293.0813.



(2S,3R,4R,5R,6S)-5-(benzyloxy)-4-hydroxy-6-methyl-2-(p-tolylthio)tetrahydro-2H-pyran-3-yl acetate (9).

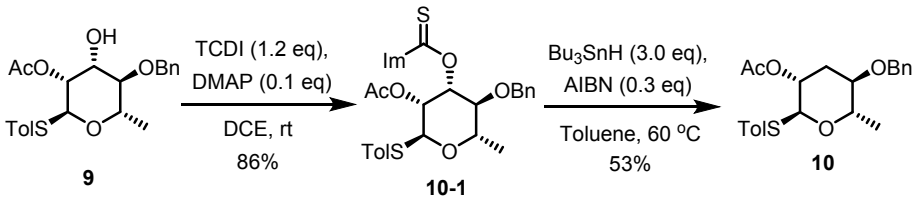
To a solution of **8** (4.3 g, 16.0 mmol) in dry CH_3CN (80 mL) at 50 °C were added triethylorthoacetate (6.1 mL, 32.1 mmol) and *p*-TsOH (552.3 mg, 3.2 mmol)

successively. The mixture was stirred at 50 °C for 8 h until no starting material was detected according to TLC. Upon completion of the reaction, Et_3N (438 μL, 3.2 mmol) was added to neutralize the solution. Then NaH (1.9 g, 48 mmol) was added, followed by BnBr (5.69 mL, 48 mmol). The resulting mixture was stirred at room temperature for 1.5 h until all the starting material was consumed by TLC. MeOH (10 mL) was carefully added to quench excess NaH. And the mixture was diluted with DCM (150 mL). The organic layer was washed with 1.0 mol/L aqueous HCl solution (50 mL × 3), saturated aqueous $NaHCO_3$ solution (100 mL × 3) and water (100 mL × 3) successively, and was then dried over anhydrous $NaSO_4$, filtered, concentrated in vacuum. The crude residue was purified by silica gel column chromatography (petrol ether : ethyl acetate = 4 : 1) to afford **9** (4.6 g, 11.4 mmol, 71%) as yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45 – 7.33 (m, 7H), 7.14 (d, *J* = 7.9 Hz, 2H), 5.39 (s, 2H), 4.90 (d, *J* = 11.1 Hz, 1H), 4.77 (d, *J* = 11.1 Hz, 1H), 4.28 (dq, *J* = 12.4, 6.2 Hz, 1H), 4.13 (dd, *J* = 10.7, 5.8 Hz, 1H), 3.48 (t, *J* = 9.4 Hz, 1H), 2.36 (s, 3H), 2.18 (s, 3H), 1.41 (d, *J* = 6.2 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 170.8, 138.2, 137.9, 132.4, 130.1, 129.9, 128.6, 128.0, 127.9, 86.3, 81.9, 75.3, 74.4, 70.7, 68.7, 21.2, 21.1, 17.8.

HR-ESI-MS (m/z) calcd for $C_{22}H_{26}NaO_5S^+$ $[M+Na]^+$ 425.1393, found 425.1393.



(2S,3R,5R,6S)-5-(benzyloxy)-6-methyl-2-(p-tolylthio)tetrahydro-2H-pyran-3-yl acetate (10).

Step 1: To a solution of **9** (3.9 g, 9.7 mmol) and TCDI (2.1 g, 11.8 mmol) in DCE (40 mL) at room temperature was added DMAP (119.7 mg, 0.98 mmol) in one portion. The mixture was stirred at room temperature for 8 h until no starting material was detected by TLC. The solvent was then removed in vacuum and the residue was purified by silica gel column chromatography (petrol ether : ethyl acetate = 4 : 1) to

afford **10-1** (4.3 g, 8.8 mmol, 86%) as white solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.18 (s, 1H), 7.54 (s, 1H), 7.41 (d, *J* = 8.0 Hz, 2H), 7.30 – 7.22 (m, 5H), 7.15 (d, *J* = 7.9 Hz, 2H), 7.04 (s, 1H), 6.15 (dd, *J* = 9.5, 3.3 Hz, 1H), 5.71 (d, *J* = 1.6 Hz, 1H), 5.43 (s, 1H), 4.75 (d, *J* = 11.4 Hz, 1H), 4.65 (d, *J* = 11.4 Hz, 1H), 4.46 (dq, *J* = 12.4, 6.2 Hz, 1H), 3.87 (t, *J* = 9.4 Hz, 1H), 2.35 (s, 3H), 2.12 (s, 3H), 1.47 (d, *J* = 6.2 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 182.4, 169.6, 138.3, 137.3, 136.4, 132.7, 131.0, 130.0, 129.1, 128.5, 128.1, 127.6, 118.0, 85.8, 80.1, 79.1, 75.3, 70.9, 69.3, 21.2, 20.9, 18.0.

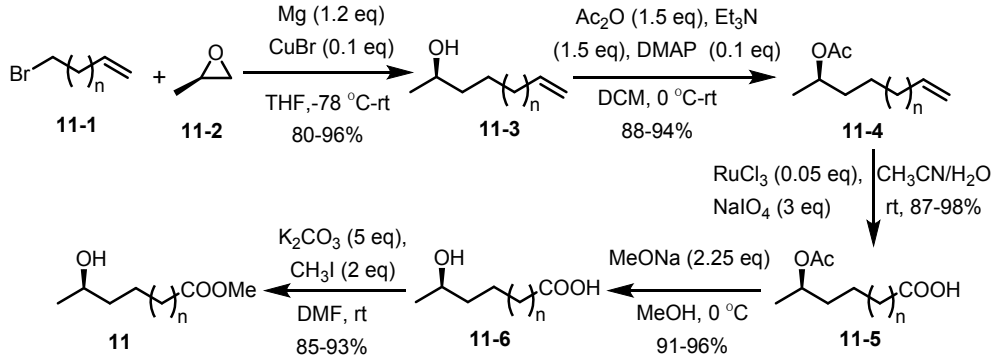
HR-ESI-MS (m/z) calcd for $C_{26}H_{29}N_2O_5S_2^+$ $[M+H]^+$ 513.1512, found 513.1513.

Step 2: To a solution of AIBN (256.9 mg, 1.5 mmol) and Bu_3SnH (4.03 mL, 15 mmol) in toluene (33 mL) at 60 °C under N_2 was added a solution of **10-1** (2.7 g，5.2 mmol) in toluene (17 mL) dropwise. The resulting mixture was stirred at 60 °C for 1.5 h until the reaction was completed as indicated by TLC. The solvent was then removed in vacuum and the residue was purified by silica gel column chromatography (petrol ether : ethyl acetate = 15: 1) to afford **10** (1.1 g, 2.8 mmol, 53%) as colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.27 (m, 7H), 7.14 (d, *J* = 7.9 Hz, 2H), 5.32 (s, 1H), 5.25 (s, 1H), 4.67 (d, *J* = 11.4 Hz, 1H), 4.54 (d, *J* = 11.4 Hz, 1H), 4.28 (dq, *J* = 12.4, 6.2 Hz, 1H), 3.53 – 3.44 (m, 1H), 2.35 – 2.31 (m, 4H), 2.10 (s, 3H), 1.95 (ddd, *J* = 14.0, 11.2, 3.0 Hz, 1H), 1.35 (d, *J* = 6.2 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.1, 138.2, 137.7, 132.4, 130.3, 129.8, 128.5, 127.8, 127.8, 86.2, 75.3, 71.9, 71.3, 69.1, 30.5, 21.2, 21.1, 18.0.

HR-ESI-MS (m/z) calcd for $C_{22}H_{26}NaO_4S^+$ $[M+Na]^+$ 409.1444, found 409.1440.



Typical procedure for the synthesis of fatty acid side chains 11a-11c (exemplified

by the synthesis of 11b).

Step 1: To a suspension of Mg (577.7 mg, 23.5 mmol) in dry THF (3 mL) at room temperature was added a solution of 7-bromo-1-heptene (3.58 mL, 23.5 mmol) in dry THF (20 mL) dropwise over 30 min. The resulting mixture was stirred at room temperature for another 3 h. Then the mixture was added dropwise to a suspension of (*R*)-(+)-1,2-epoxypropane (1.32 mL, 19.0 mmol) and CuBr (272 mg, 1.9 mmol) in dry THF (23 mL) at -78 °C under argon. Upon the completion of the addition, the mixture was warmed to room temperature and stirred for 10 h until the reaction was completed according to TLC. The reaction was quenched by saturated aqueous NH_4Cl solution (20 mL) and extracted by Et_2O (30 mL×3). The organic layers were dried by anhydrous Na_2SO_4, and concentrated in vacuum. The crude product was purified by silica gel column chromatography (n-pentane : Et_2O = 4 : 1) to afford **11b-3** (2.4 g, 15.4 mmol, 80%) as colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 5.83 – 5.72 (m, 1H), 4.96 (d, *J* = 17.1 Hz, 1H), 4.90 (d, *J* = 10.2 Hz, 1H), 3.78 – 3.70 (m, 1H), 2.55 – 2.41 (m, 1H), 2.06 – 1.97 (m, 2H), 1.40 – 1.23 (m, 9H), 1.14 (d, *J* = 6.2 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 139.1, 114.1, 67.9, 39.2, 33.8, 29.5, 29.1, 28.8, 25.7, 23.3.

Step 2: To a stirred solution of **11b-3** (2.3 g, 14.7 mmol) and DMAP (91.8 mg, 1.5 mmol) in DCM (50 mL) at 0 °C were added Et_3N (3.1 mL, 22.3 mmol) and Ac_2O (2.1 mL, 22.3 mmol) successively. The mixture was stirred at room temperature for 4 h until completion. The reaction was quenched by saturated aqueous $NaHCO_3$ solution (20 mL) and extracted with DCM (50 mL × 3). The organic layers were washed with brine (50 mL × 3), dried over anhydrous $NaSO_4$, filtered, and concentrated in vacuum. The crude product was purified by silica gel column chromatography (petrol ether : ethyl acetate = 15 : 1) to afford **11b-4** (2.7 g, 13.6 mmol, 93%) as colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.81 (ddt, *J* = 17.0, 10.2, 6.7 Hz, 1H), 5.03 – 4.92 (m, 2H), 4.88 (dd, *J* = 12.7, 6.5 Hz, 1H), 2.09 – 2.00 (m, 5H), 1.64 – 1.53 (m, 1H), 1.51 – 1.43 (m, 1H), 1.41 – 1.34 (m, 2H), 1.28 (d, *J* = 14.3 Hz, 6H), 1.20 (t, *J* = 6.1 Hz,

3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.8, 139.1, 114.2, 71.0, 35.9, 33.8, 29.3, 29.0, 28.8, 25.4, 21.4, 20.0.

Step 3: To a solution of **11b-4** (2.9 g, 14.7 mmol) in CH_3CN (15 mL) and H_2O (7 mL) was added an aqueous solution of $RuCl_3 \cdot 3H_2O$ (192.1 mg, 0.7 mmol) in H_2O (8 mL). The mixture was stirred at room temperature vigorously. After 5 min, sodium periodate (9.4 g, 44 mmol) was added. The resulting mixture was stirred at room temperature overnight until the reaction was completed according to TLC. The mixture was diluted with H_2O (20 mL) and extracted with DCM (50 mL × 3). The organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuum. The crude product was purified by silica gel column chromatography (petrol ether : ethyl acetate = 4 : 1) to afford **11b-5** (2.7 g, 12.5 mmol, 87%) as yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.93 – 4.83 (m, 1H), 2.35 (t, J = 7.5 Hz, 2H), 2.03 (s, 3H), 1.69 – 1.52 (m, 3H), 1.51 – 1.40 (m, 1H), 1.41 – 1.25 (m, 6H), 1.20 (d, J = 6.3 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 179.8, 171.0, 71.1, 35.8, 34.0, 29.0, 28.9, 25.2, 24.6, 21.4, 19.9.

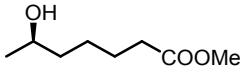
Step 4: To a stirred solution of compound **11b-5** (2.5 g, 11.6 mmol) in MeOH (20 mL) at 0 °C was added a freshly prepared solution of MeONa (1.4 g, 26.1 mmol) in MeOH (20 mL). The solution was warmed to room temperature and was stirred for 4 h. The reaction was neutralized by acidic Amberlite IR-120 (H^+), filtered and concentrated in vacuum. The crude product was purified by silica gel column chromatography (petrol ether : ethyl acetate = 1 : 1) to afford **11b-6** (1.9 g, 10.9 mmol, 96%) as colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.87 – 3.73 (m, 1H), 2.40 – 2.25 (m, 2H), 1.67 – 1.57 (m, 2H), 1.53 – 1.25 (m, 8H), 1.18 (d, J = 6.2 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 178.9, 68.2, 39.0, 34.0, 29.2, 29.0, 25.5, 24.6, 23.2

Step 5: To a mixture of **11b-6** (1.7 g, 9.8 mmol) and K_2CO_3 (6.8 g, 49.5 mmol) in DMF (40 mL) was added MeI (1.23 mL, 19.8 mmol) and the resulting mixture was

stirred at room temperature for 10 h. The reaction was quenched with saturated aqueous NH_4Cl solution (20 mL) and the mixture was extracted with ethyl acetate (50 mL × 3). The organic layers were washed with brine (50 mL × 3), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuum. The crude product was purified by silica gel column chromatography (petrol ether : ethyl acetate = 4 : 1) to afford **11b** (1.7 g, 9.0 mmol, 90%) as colorless oil.



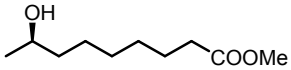
11a

(R)-methyl-6-hydroxyheptanoate (11a).

The spectral data of **11a** are identical to those of a previous report.[4]

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.82 – 3.73 (m, 1H), 3.64 (s, 3H), 2.30 (t, J = 7.5 Hz, 2H), 2.03 (s, 1H), 1.68 – 1.56 (m, 2H), 1.49 – 1.28 (m, 4H), 1.15 (d, J = 6.2 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 174.3, 67.7, 51.5, 38.8, 34.0, 25.3, 24.8, 23.4.



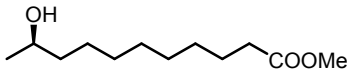
11b

(R)-methyl -8-hydroxynonanoate (11b).

The spectral data of **11b** are identical to those of a previous report.[4]

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.75 – 3.63 (m, 1H), 3.59 (s, 3H), 2.39 (s, 1H), 2.23 (t, J = 7.5 Hz, 2H), 1.61 – 1.50 (m, 2H), 1.43 – 1.18 (m, 8H), 1.10 (dd, J = 6.1, 2.0 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 174.3, 67.6, 51.4, 39.1, 33.9, 29.2, 29.0, 25.5, 24.7, 23.3.



11c

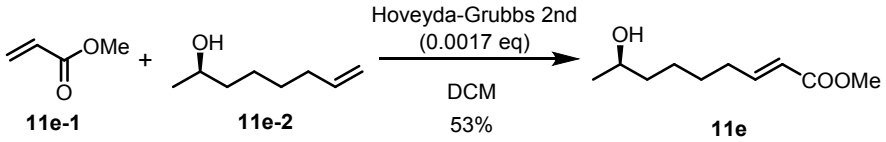
(R)-methyl -10-hydroxyundecanoate(11c).

The spectral data of **11c** are identical to those of a previous report.[5]

^{1}H NMR (500 MHz, $CDCl_3$) δ 3.79 – 3.70 (m, 1H), 3.63 (s, 3H), 2.27 (t, J = 7.6 Hz, 2H), 1.91 (s, 1H), 1.62 – 1.54 (m, 2H), 1.47 – 1.34 (m, 3H), 1.32 – 1.23 (m, 9H), 1.14 (d, J = 6.2 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 174.4, 68.0, 51.4, 39.3, 34.0, 29.5, 29.4, 29.1, 29.1, 25.7, 24.9, 23.4.

Typical procedure for the synthesis of fatty acid side chains 11e.



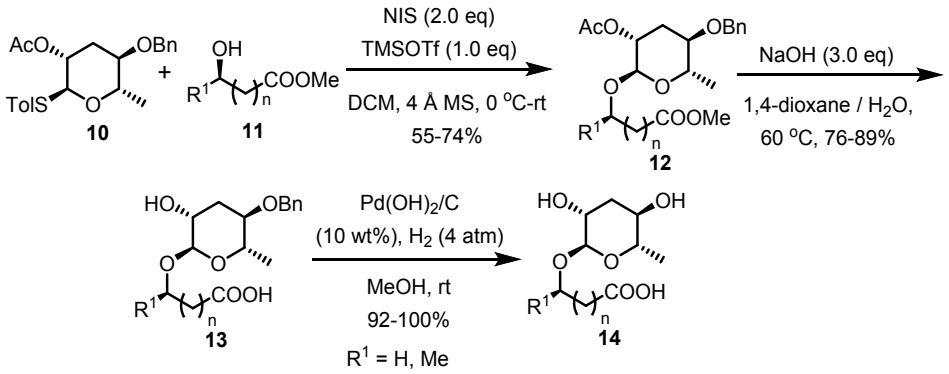
To a solution of methyl acrylate (**11e-1**, 809 μL, 6 mmol) and **11e-2** (256 mg, 2 mmol) in DCM (5 mL) was added Hoveyda-Grubbs 2nd generation catalyst (8.5 mg, 0.01 mmol), and the reaction was allowed to stir at room temperature for 10 h. The solvent was then removed in vacuum and the residue was purified by silica gel column chromatography (petrol ether : ethyl acetate = 4 : 1) to afford **11e** (197 mg, 1.1 mmol, 53%) as colorless oil.

The spectral data of **11e** are identical to those of a previous report.[6]

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.95 (dt, *J* = 15.6, 7.0 Hz, 1H), 5.81 (dt, *J* = 15.6, 1.5 Hz, 1H), 3.80 – 3.73 (m, 1H), 3.71 (s, 3H), 2.20 (qd, *J* = 7.1, 1.2 Hz, 2H), 1.51 – 1.38 (m, 5H), 1.36 – 1.30 (m, 1H), 1.17 (d, *J* = 6.2 Hz, 3H).

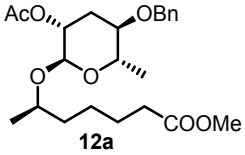
^{13}C NMR (126 MHz, $CDCl_3$) δ 167.2, 149.5, 120.9, 67.8, 51.4, 39.0, 32.2, 28.0, 25.3, 23.5.

Typical procedure for the preparation of ascarosides 14a-14d (exemplified by the synthesis of 14a).



Step 1: To a suspension of **10** (100 mg, 0.26 mmol), **11a** (41.5 mg, 0.52 mmol), NIS (117 mg, 0.52 mmol), and 4 Å molecular sieves in dry DCM (2 mL) was added TMSOTf (14 μL, 0.078 mmol) slowly under N_2 at room temperature. The mixture was stirred at room temperature for 1 h. Et_3N (36 μL, 0.26 mmol) was added to quench the reaction. The resulting mixture was filtered, and concentrated in vacuum.

The residue was purified by silica gel column chromatography (petrol ether : ethyl acetate = 15 : 1) to afford **12a** (60 mg, 0.14 mmol, 55%) as yellow oil.



12a

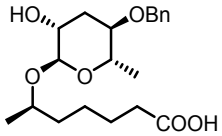
(R)-Methyl--6-(((2R,3R,5R,6S)-3-acetoxy-5-(benzyloxy)-6-methyltetrahydro-2H-pyran-2-yl)oxy)heptanoate (12a).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 – 7.29 (m, 5H), 4.86 (s, 1H), 4.72 (s, 1H), 4.63 (d, *J* = 11.4 Hz, 1H), 4.49 (d, *J* = 11.4 Hz, 1H), 3.85 – 3.75 (m, 2H), 3.66 (s, 3H), 3.37 (td, *J* = 10.8, 4.3 Hz, 1H), 2.33 (t, *J* = 7.5 Hz, 2H), 2.22 (dt, *J* = 13.6, 3.6 Hz, 1H), 2.09 (s, 3H), 1.94 – 1.87 (m, 1H), 1.70 – 1.60 (m, 2H), 1.60 – 1.53 (m, 1H), 1.50 – 1.41 (m, 2H), 1.40 – 1.33 (m, 1H), 1.30 (d, *J* = 6.2 Hz, 3H), 1.12 (d, *J* = 6.1 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 174.1, 170.3, 138.2, 128.4, 127.8, 127.76, 93.5, 75.1, 71.7, 71.2, 68.2, 51.5, 36.7, 34.0, 29.4, 25.2, 24.9, 21.2, 18.9, 18.0.

HR-ESI-MS (m/z) calcd for $C_{23}H_{34}NaO_7^+$ $[M+Na]^+$ 445.2197, found 445.2197.

Step 2: To a solution of **12a** (159.6 mg, 0.39 mmol) in 1,4-dioxane (4 mL) was added a solution of NaOH (46.8 mg, 1.17 mmol) in H_2O (0.5 mL). The resulting mixture was stirred at 60 °C for 3 h until no starting material was detected by TLC. The aqueous layer was acidified to *p*H 2 with aqueous HCl solution (1.0 mol/L), and was extracted with Et_2O (10 mL × 2). The corganic layers were washed with brine (10 mL × 2), dried over anhydrous Na_2SO_4, and concentrated in vacuum. The crude product was purified by silica gel column chromatography (DCM : MeOH = 15 : 1) to afford **13a** (105 mg, 0.29 mmol, 76%) as colorless oil.



13a

(R)-6-(((2R,3R,5R,6S)-5-(benzyloxy)-3-hydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)heptanoic acid (13a).

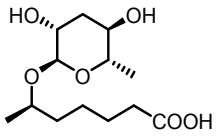
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 – 7.29 (m, 5H) 4.71 (s, 1H), 4.64 (d, *J* = 11.5 Hz, 1H), 4.49 (d, *J* = 11.5 Hz, 1H), 3.86 – 3.77 (m, 3H), 3.42 (td, *J* = 10.6, 4.3 Hz, 1H),

2.36 (t, J = 7.4 Hz, 2H), 2.25 – 2.19 (m, 1H), 1.90 – 1.81 (m, 1H), 1.70 – 1.54 (m, 3H), 1.52 – 1.37 (m, 3H), 1.29 (d, J = 6.3 Hz, 3H), 1.14 (d, J = 6.0 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 178.8, 138.2, 128.5, 127.9, 127.8, 96.1, 75.2, 71.4, 71.1, 69.1, 68.4, 36.7, 33.9, 31.8, 25.1, 24.7, 18.9, 18.1.

HR-ESI-MS (m/z) calcd for $C_{20}H_{30}NaO_6^+$ $[M+Na]^+$ 389.1935, found 389.1937.

Step 3: A mixture of **13a** (47.5 mg, 0.13 mmol) and $Pd(OH)_2/C$ (18.2 mg, 0.013 mmol) in MeOH (2 mL) under H_2 (4 atm) was stirred at room temperature for 12 h. Then the reaction mixture was filtered and was concentrated in vacuum. The residue was purified by flash column chromatography (DCM : MeOH = 15:1) to afford **14a/7** (35 mg, 0.13 mmol, 100%) as a colourless oil.



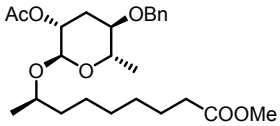
14a/7

6R-(3'R,5'R-dihydroxy-6'S-methyl-(2H)-tetrahydropyran-2'-yloxy) heptanoic acid (14a/7, ascr#1)[2]

^{1}H NMR (400 MHz, CD_3OD) δ 4.66 (s, 1H), 3.85 – 3.76 (m, 1H), 3.74 (d, J = 0.9 Hz, 1H), 3.67 – 3.59 (m, 1H), 3.57 – 3.47 (m, 1H), 2.32 (t, J = 7.4 Hz, 2H), 2.02 – 1.92 (m, 1H), 1.83 – 1.73 (m, 1H), 1.70 – 1.56 (m, 3H), 1.55 – 1.39 (m, 3H), 1.23 (d, J = 6.2 Hz, 3H), 1.14 (d, J = 6.1 Hz, 3H);

^{13}C NMR (101 MHz, CD_3OD) δ 177.6, 97.4, 72.2, 71.1, 69.9, 68.3, 38.0, 35.9, 34.8, 26.3, 26.0, 19.3, 18.0;

HR-ESI-MS (m/z) calcd for $C_{13}H_{24}NaO_6^+$ $[M+Na]^+$ 299.1465, found 299.1464.



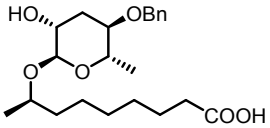
12b

(R)-Methyl--8-(((2R,3R,5R,6S)-3-acetoxy-5-(benzyloxy)-6-methyltetrahydro-2H-pyran-2-yl)oxy)nonanoate (12b).

12b (99.8 mg, 0.22 mmol, 61%) was obtained from **10** (151 mg, 0.39 mmol) and **11b** (88 mg, 0.47 mmol) as yellow oil.

^{1}**H NMR** (500 MHz, $CDCl_3$) δ 7.36 – 7.29 (m, 5H), 4.85 (s, 1H), 4.71 (s, 1H), 4.61 (d, *J* = 11.4 Hz, 1H), 4.47 (d, *J* = 11.4 Hz, 1H), 3.85 – 3.78 (m, 1H), 3.75 (dd, *J* = 11.4, 5.8 Hz, 1H), 3.65 (s, 3H), 3.35 (td, *J* = 10.4, 4.2 Hz, 1H), 2.29 (t, *J* = 7.5 Hz, 2H), 2.23 – 2.17 (m, 1H), 2.07 (s, 3H), 1.90 (dd, *J* = 17.9, 6.6 Hz, 1H), 1.64 – 1.58 (m, 2H), 1.45 – 1.36 (m, 2H), 1.34 – 1.29 (m, 6H), 1.28 (d, *J* = 6.3 Hz, 3H), 1.10 (d, *J* = 6.0 Hz, 3H).

^{13}**C NMR** (126 MHz, $CDCl_3$) δ 174.2, 170.3, 138.2, 128.4, 127.8, 127.7, 93.5, 75.1, 71.8, 71.2, 71.2, 68.1, 51.4, 37.0, 34.1, 29.2, 29.0, 25.5, 25.1, 24.9, 21.2, 19.0, 18.0.



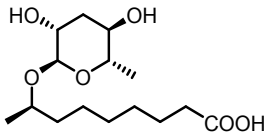
13b

(R)-8-(((2R,3R,5R,6S)-5-(benzyloxy)-3-hydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)nonanoic acid (13b).

13b (35 mg, 0.089 mmol, 89%) was obtained from **12b** (45 mg, 0.1 mmol) as yellow oil.

^{1}**H NMR** (500 MHz, $CDCl_3$) δ 7.39 – 7.30 (m, 5H), 4.72 (s, 1H), 4.64 (d, *J* = 11.5 Hz, 1H), 4.49 (d, *J* = 11.5 Hz, 1H), 3.88 – 3.83 (m, 2H), 3.79 (dd, *J* = 11.9, 6.1 Hz, 1H), 3.46 – 3.39 (m, 1H), 2.34 (t, *J* = 7.5 Hz, 2H), 2.22 (dt, *J* = 13.1, 3.9 Hz, 1H), 1.90 – 1.83 (m, 1H), 1.68 – 1.61 (m, 2H), 1.47 – 1.38 (m, 2H), 1.31 (m, 6H), 1.28 (s, 3H), 1.13 (d, *J* = 6.1 Hz, 3H).

^{13}**C NMR** (126 MHz, $CDCl_3$) δ 179.1, 138.1, 128.4, 127.8, 127.7, 96.2, 75.2, 71.7, 71.1, 69.1, 68.4, 37.2, 33.9, 31.8, 29.5, 29.4, 25.6, 24.7, 18.9, 18.0.



14b

(R)-8-(((2R,3R,5R,6S)-3,5-dihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy) nonanoic acid (14b,* ascr#10*).

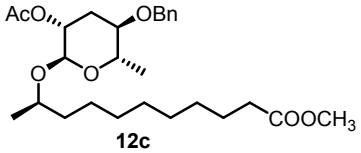
14b (13 mg, 0.043 mmol, 96%) was obtained from **13b** (18 mg, 0.046 mmol) as yellow oil.

The spectral data of **14b** are identical to those of a previous report.[7]

^{1}H NMR (400 MHz, CD_3OD) δ 4.66 (s, 1H), 3.85 – 3.75 (m, 1H), 3.73 (d, *J* = 0.8 Hz, 1H), 3.65 (dq, *J* = 9.6, 6.1 Hz, 1H), 3.58 – 3.49 (m, 1H), 2.30 (t, *J* = 7.4 Hz, 2H), 1.97 (dt, *J* = 12.9, 3.6 Hz, 1H), 1.83 – 1.74 (m, 1H), 1.68 – 1.53 (m, 3H), 1.52 – 1.43 (m, 2H), 1.43 – 1.32 (m, 5H), 1.23 (d, *J* = 6.2 Hz, 3H), 1.14 (d, *J* = 6.1 Hz, 3H).

^{13}C NMR (101 MHz, CD_3OD) δ 178.5, 98.1, 73.1, 71.8, 70.6, 68.9, 38.9, 36.6, 35.7, 31.0, 30.8, 27.4, 26.7, 20.0, 18.7.

HR-ESI-MS (m/z) calcd for $C_{15}H_{28}NaO_6^+$ $[M+Na]^+$ 327.1778, found 327.1774.



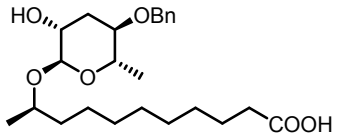
12c

(R)-Methyl-10-(((2R,3R,5R,6S)-3-acetoxy-5-(benzyloxy)-6-methyltetrahydro-2H-pyran-2-yl)oxy)undecanoate (12c).

12c (138 mg, 0.29 mmol, 74%) was obtained from **10** (151 mg, 0.39 mmol) and **11c** (102 mg, 0.47 mmol) as yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.30 (m, 5H), 4.87 (d, *J* = 1.0 Hz, 1H), 4.73 (s, 1H), 4.63 (d, *J* = 11.4 Hz, 1H), 4.48 (d, *J* = 11.4 Hz, 1H), 3.89 – 3.74 (m, 2H), 3.66 (s, 3H), 3.37 (ddd, *J* = 11.2, 9.6, 4.3 Hz, 1H), 2.30 (t, *J* = 7.5 Hz, 2H), 2.22 (dt, *J* = 7.2, 3.8 Hz, 1H), 2.08 (s, 3H), 1.91 (ddd, *J* = 13.9, 11.3, 3.0 Hz, 1H), 1.66 – 1.58 (m, 2H), 1.45 – 1.39 (m, 2H), 1.33 – 1.28 (m, 10H), 1.28 (d, *J* = 8.0 Hz, 3H), 1.12 (d, *J* = 6.1 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 174.3, 170.3, 138.2, 128.4, 127.8, 127.7, 93.5, 75.1, 71.9, 71.3, 71.2, 68.1, 51.4, 37.1, 34.1, 29.5, 29.4, 29.4, 29.2, 29.1, 25.6, 24.9, 21.2, 19.0, 18.0.



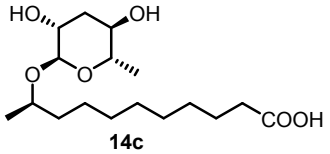
13c

(R)-10-(((2R,3R,5R,6S)-5-(benzyloxy)-3-hydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)undecanoic acid (13c).

13c (50 mg, 0.12 mmol, 82%) was obtained from **12c** (69 mg, 0.14 mmol) as yellow oil.

[1]**H NMR** (500 MHz, $CDCl_3$) δ 7.37 – 7.29 (m, 5H), 4.72 (s, 1H), 4.64 (d, *J* = 11.5 Hz, 1H), 4.49 (d, *J* = 11.5 Hz, 1H), 3.87 – 3.82 (m, 2H), 3.79 (td, *J* = 12.1, 6.0 Hz, 1H), 3.46 – 3.39 (m, 1H), 2.34 (t, *J* = 7.5 Hz, 2H), 2.22 (dt, *J* = 13.1, 3.9 Hz, 1H), 1.90 – 1.82 (m, 1H), 1.67 – 1.60 (m, 2H), 1.46 – 1.38 (m, 2H), 1.36 – 1.29 (m, 10H), 1.29 (d, *J* = 6.3 Hz, 3H), 1.13 (d, *J* = 6.1 Hz, 3H).

[13]**C NMR** (126 MHz, $CDCl_3$) δ 179.1, 138.3, 128.4, 127.8, 127.7, 96.2, 75.2, 71.7, 71.1, 69.1, 68.4, 37.2, 34.0, 31.8, 29.5, 29.2, 29.1, 25.6, 24.7, 19.0, 18.0.



14c

(R)-10-(((2R,3R,5R,6S)-3,5-dihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)undecanoic acid (14c* , ascr#18*).

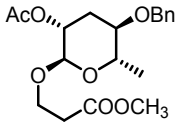
14c (17 mg, 0.05 mmol, 92%) was obtained from **13c** (25 mg, 0.06 mmol) as yellow oil.

The spectral data of **14c** are identical to those of a previous report.[8]

[1]**H NMR** (500 MHz, CD_3OD) δ 4.66 (s, 1H), 3.86 – 3.76 (m, 1H), 3.74 (d, *J* = 1.0 Hz, 1H), 3.65 (dq, *J* = 9.5, 5.9 Hz, 1H), 3.58 – 3.49 (m, 1H), 2.28 (t, *J* = 7.4 Hz, 2H), 1.97 (dt, *J* = 13.0, 3.7 Hz, 1H), 1.84 – 1.74 (m, 1H), 1.67 – 1.53 (m, 3H), 1.51 – 1.43 (m, 2H), 1.41 – 1.31 (m, 9H), 1.23 (d, *J* = 6.2 Hz, 3H), 1.13 (d, *J* = 6.1 Hz, 3H).

[13]**C NMR** (126 MHz, CD_3OD) δ 177.3, 96.2, 71.1, 69.8, 68.6, 66.9, 37.0, 34.6, 34.3, 29.3, 29.2, 29.1, 29.0, 25.5, 25.0, 18.0, 16.7.

HR-ESI-MS (m/z) calcd for $C_{17}H_{32}NaO_6^+$ $[M+Na]^+$ 355.2091, found 355.2091.



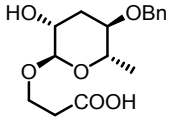
12d

(2R,3R,5R,6S)-5-(benzyloxy)-6-methyl-2-(3-(methylperoxy)-3-oxopropoxy)tetrahydro-2H-pyran-3-yl acetate (12d).

12d (81 mg, 0.22 mmol, 57%) was obtained from **10** (151 mg, 0.39 mmol) and **11d** (81 mg, 0.78 mmol) as yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 – 7.31 (m, 5H), 4.91 (s, 1H), 4.62 (d, *J* = 12.5 Hz, 2H), 4.48 (d, *J* = 11.5 Hz, 1H), 3.99 (dt, *J* = 9.8, 6.4 Hz, 1H), 3.82 – 3.76 (m, 1H), 3.74 (d, *J* = 6.2 Hz, 1H), 3.71 (s, 3H), 3.36 (td, *J* = 10.5, 4.3 Hz, 1H), 2.61 (t, *J* = 6.3 Hz, 2H), 2.24 – 2.17 (m, 1H), 2.09 (s, 3H), 1.92 – 1.84 (m, 1H), 1.32 (d, *J* = 6.2 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 171.7, 170.2, 138.2, 129.8, 128.4, 127.7, 96.4, 74.9, 71.1, 70.4, 68.1, 62.9, 51.8, 34.6, 29.3, 21.2, 18.0.



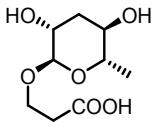
13d

3-(((2R,3R,5R,6S)-5-(benzyloxy)-3-hydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)propanoic acid (13d).

13d (26 mg, 0.084 mmol, 76%) was obtained from **12d** (40 mg, 0.11 mmol) as yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 – 7.32 (m, 5H), 4.65 – 4.59 (m, 2H), 4.47 (d, *J* = 11.5 Hz, 1H), 4.01 – 3.94 (m, 1H), 3.87 (s, 1H), 3.80 (td, *J* = 12.6, 6.3 Hz, 1H), 3.76 – 3.70 (m, 1H), 3.43 (td, *J* = 10.5, 4.4 Hz, 1H), 2.63 (t, *J* = 5.8 Hz, 2H), 2.18 (dt, *J* = 12.9, 3.5 Hz, 1H), 1.87 – 1.78 (m, 1H), 1.30 (d, *J* = 6.2 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 176.4, 138.2, 128.4, 127.8, 127.7, 99.2, 75.0, 71.1, 68.4, 68.2, 62.7, 31.6, 18.0.



14d

3-(((2R,3R,5R,6S)-3,5-dihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)propanoic acid (14d, **ascr#5*)***.

14d (9 mg, 0.04 mmol, 97%) was obtained from **13d** (13 mg, 0.042 mmol) as yellow oil.

The spectral data of **14d** are identical to those of a previous report.[9]

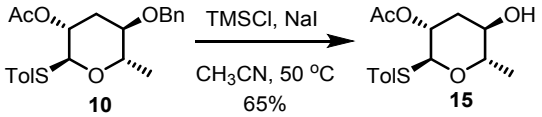
^{1}H NMR (400 MHz, CD_3OD) δ 4.56 (s, 1H), 3.96 (dt, *J* = 9.7, 6.5 Hz, 1H), 3.77 (d, *J* = 1.3 Hz, 1H), 3.70 (dt, *J* = 9.7, 6.5 Hz, 1H), 3.65 – 3.58 (m, 1H), 3.56 – 3.48 (m,

1H), 2.51 (t, J = 6.5 Hz, 2H), 1.94 (dt, J = 13.0, 3.6 Hz, 1H), 1.77 (ddd, J = 13.2, 11.3, 3.1 Hz, 1H), 1.25 (d, J = 6.1 Hz, 3H).

^{13}C NMR (101 MHz, CD_3OD) δ 169.1,99.0, 69.5, 67.9, 66.9, 63.7, 36.7, 34.5, 16.7.

HR-ESI-MS (m/z) calcd for $C_9H_{16}NaO_6^+$ $[M+Na]^+$ 243.0839, found 243.0841.

The procedure for preparation of (2S,3R,5R,6S)-5-hydroxy-6-methyl-2-(p-tolyl thio) tetrahydro-2H-pyran-3-yl acetate (15).



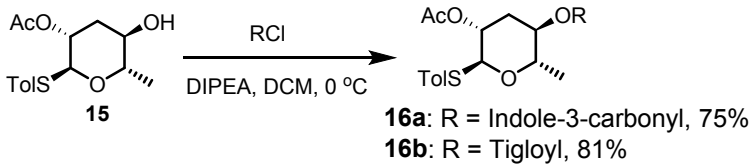
To a solution of **10**（78 mg, 0.2 mmol) and NaI (60 mg, 0.4 mmol) in dry CH_3CN (2 mL) was slowly added TMSCl (40 μL, 0.4 mmol) at room temperature. Then the mixture was stirred at 50 °C for 10 h. The reaction was diluted with H_2O (10 mL), extracted with ethyl acetate (20 mL × 3). The organic layers were washed with brine (20 mL × 3), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuum. The crude product was purified by silica gel column chromatography (petrol ether: ethyl acetate = 4 : 1) to afford **15** (38 mg, 0.13 mmol, 65%) as colorless oil with conversion ratio of 75%.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 (d, J = 7.3 Hz, 2H), 7.14 (d, J = 7.6 Hz, 2H), 5.32 (s, 1H), 5.22 (s, 1H), 4.89 (td, J = 10.4, 4.5 Hz, 1H), 4.32 (dq, J = 12.3, 6.2 Hz, 1H), 2.34 (s, 3H), 2.25 (d, J = 13.7 Hz, 1H), 2.12 (s, 3H), 2.10 (s, 3H), 1.96 (t, J = 12.4 Hz, 1H), 1.23 (d, J = 6.1 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 170.3, 137.8, 132.3, 130.1, 129.9, 86.1, 72.1, 70.5, 68.2, 33.5, 21.2, 21.2, 17.6.

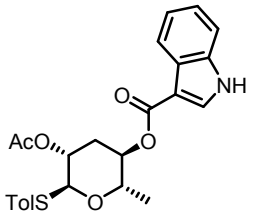
HRMS (ESI) m/z calcd for $C_{15}H_{20}NaO_4S^+$ $[M+Na]^+$ 319.0974, found 319.0975.

The procedure for preparation of 16a-16b (exemplified by 16a).



To a solution of Indole-3-carboxylic acid (239.7 mg, 0.81 mmol) in dry DCM (5 mL) with a drop of DMF at 0 °C was slowly added oxalyl chloride (137 μL, 1.62 mmol).

The resulting solution was stirred for 20 min at room temperature until the starting material was consumed according to TLC. The reaction mixture was concentrated to dryness in vacuum. Then the residue was redissolved in dry DCM (2 mL). The resulting solution was added dropwise to a stirred solution of **15** (240 mg, 0.81 mmol) and *N,N*-diisopropylethylamine (DIPEA, 0.57 mL, 3.24 mmol) in dry DCM (5 mL) at 0 °C in an ice bath. After 20 min, the reaction was quenched with saturated aqueous $NaHCO_3$ solution, extracted with DCM, dried over Na_2SO_4, and concentrated in vacuum. The crude product was purified by silica gel column chromatography (petrol ether : ethyl acetate = 4 : 1) to afford **16a** (267 mg, 0.61 mmol, 75%) as white solid.



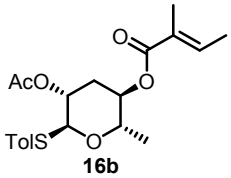
16a

(2S,3R,5R,6S)-5-acetoxy-2-methyl-6-(p-tolylthio)tetrahydro-2H-pyran-3-yl-1H-indole-3-carboxylate (16a).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.97 (s, 1H), 8.22 – 8.17 (m, 1H), 7.96 (d, *J* = 3.0 Hz, 1H), 7.46 (s, 1H), 7.44 (d, *J* = 8.1 Hz, 2H), 7.34 – 7.30 (m, 2H), 7.16 (d, *J* = 8.0 Hz, 2H), 5.41 (s, 1H), 5.31 (s, 1H), 5.23 (td, *J* = 10.9, 4.7 Hz, 1H), 4.59 – 4.50 (m, 1H), 2.46 (d, *J* = 13.8 Hz, 1H), 2.36 (s, 3H), 2.18 (d, *J* = 3.1 Hz, 1H), 2.15 (s, 3H), 1.36 (d, *J* = 6.2 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 170.4, 164.3, 137.9, 136.2, 132.4, 131.5, 130.0, 129.9, 125.8, 123.4, 122.3, 121.4, 111.7, 108.4, 86.3, 71.5, 69.2, 68.2, 30.9, 21.2, 21.1, 17.8.

HR-ESI-MS (m/z) calcd for $C_{24}H_{25}NNaO_5S^+$ $[M+Na]^+$ 462.1346, found 462.1343.



16b

(2S,3R,5R,6S)-5-acetoxy-2-methyl-6-(p-tolylthio)tetrahydro-2H-pyran-3-yl-(E)-2-methylbut-2-enoate (16b).

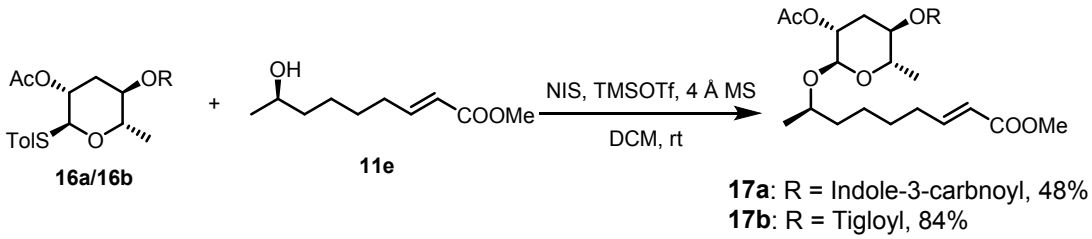
16b (173 mg, 0.46 mmol, 81%) was obtained from **15** (170 mg, 0.57 mmol) as yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 (d, *J* = 8.1 Hz, 2H), 7.13 (d, *J* = 8.0 Hz, 2H), 6.90 (qd, *J* = 6.8, 1.1 Hz, 1H), 5.33 (s, 1H), 5.23 (d, *J* = 0.6 Hz, 1H), 4.95 (td, *J* = 10.9, 4.6 Hz, 1H), 4.38 (dq, *J* = 12.3, 6.2 Hz, 1H), 2.33 (s, 3H), 2.31 – 2.26 (m, 1H), 2.11 (s, 3H), 1.97 (ddd, *J* = 14.0, 8.4, 3.1 Hz, 1H), 1.85 (s, 3H), 1.82 (d, *J* = 7.1 Hz, 3H), 1.24 (d, *J* = 6.2 Hz, 3H).

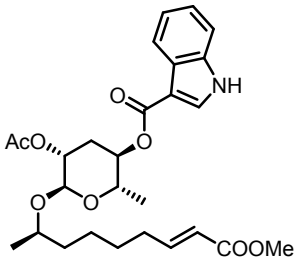
^{13}C NMR (126 MHz, $CDCl_3$) δ 170.2, 167.0, 138.0, 137.8, 132.3, 129.9, 129.9, 128.4, 86.2, 71.3, 69.8, 67.9, 30.5, 21.2, 21.1, 17.6, 14.5, 12.1.

HR-ESI-MS (m/z) calcd for $C_{20}H_{26}NaO_5S^+$ $[M+Na]^+$ 401.1393, found 401.1392.

The procedure for preparation of 17a-17b (exemplified by 17a).



To a solution of **16a** (150 mg, 0.34 mmol), **11e** (94.9 mg, 0.51 mmol) , NIS (152.9 mg , 0.68 mmol), and 4 Å molecular sieves in dry DCM (2 mL) under N_2 at room temperature was added TMSOTf (61 μL, 0.34 mmol) dropwise. The solution was stirred for 1 h. Then Et_3N (47 μ L, 0.34 mmol) was added to quench the reaction. The mixture was extracted with DCM, dried over Na_2SO_4, and concentrated in vacuum. The crude product was purified by silica gel column chromatography (petrol ether : ethyl acetate = 12 : 1) to afford **17a** (82 mg, 0.16 mmol, 48%) as yellow oil.



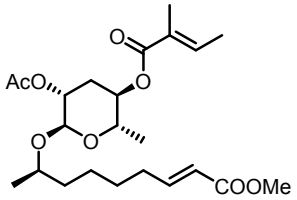
17a

(2S,3R,5R,6R)-5-acetoxy-6-(((R,E)-9-methoxy-9-oxonon-7-en-2-yl)oxy)-2-methyltetrahydro-2H-pyran-3-yl 1H-indole-3-carboxylate (17a).

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.14 (s, 1H), 8.18 – 8.10 (m, 1H), 7.95 (d, *J* = 2.7 Hz, 1H), 7.45 (dd, *J* = 6.1, 2.4 Hz, 1H), 7.32 – 7.25 (m, 2H), 7.08 – 6.98 (m, 1H), 5.88 (d, *J* = 15.6 Hz, 1H), 5.13 (td, *J* = 10.8, 4.5 Hz, 1H), 4.94 (s, 1H), 4.83 (s, 1H), 4.10 – 4.02 (m, 1H), 3.84 (d, *J* = 4.6 Hz, 1H), 3.72 (s, 3H), 2.37 – 2.25 (m, 3H), 2.15 (s, 3H), 2.11 (d, *J* = 2.6 Hz, 1H), 1.70 – 1.44 (m, 6H), 1.30 (d, *J* = 6.2 Hz, 3H), 1.18 (d, *J* = 6.0 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 170.6, 167.3, 164.3, 149.6, 136.2, 131.6, 125.8, 123.3, 122.1, 121.3, 121.0, 111.7, 108.3, 93.7, 72.2, 70.8, 69.1, 67.3, 51.5, 36.9, 32.3, 29.8, 28.0, 25.4, 21.3, 19.1, 17.9.

HR-ESI-MS (m/z) calcd for $C_{27}H_{35}NNaO_8^+$ $[M+Na]^+$ 524.2255, found 524.2256.



17b

(R,E)-Methyl-8-(((2R,3R,5R,6S)-3-acetoxy-6-methyl-5-(((E)-2-methylbut-2-enoyl)oxy)tetrahydro-2H-pyran-2-yl)oxy)non-2-enoate (17b).

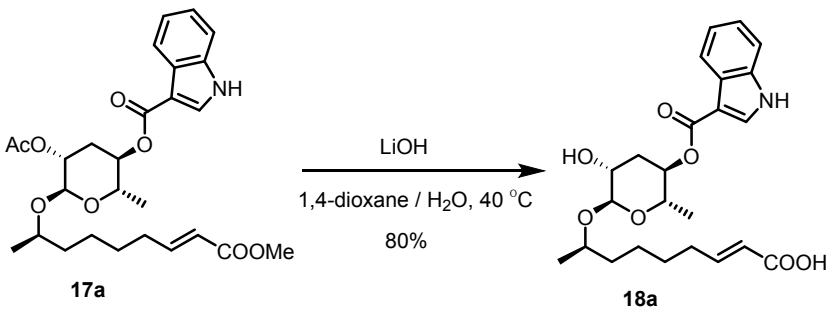
17b (85 mg, 0.19 mmol, 84%) was obtained from **16b** (86.5 mg, 0.23 mmol) as yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.97 (dt, *J* = 15.4, 7.0 Hz, 1H), 6.88 – 6.81 (m, 1H), 5.83 (d, *J* = 15.6 Hz, 1H), 4.89 – 4.79 (m, 2H), 4.75 (s, 1H), 3.90 (dq, *J* = 12.4, 6.2 Hz, 1H), 3.77 (dd, *J* = 11.4, 6.2 Hz, 1H), 3.74 – 3.68 (m, 3H), 2.23 (dd, *J* = 13.4, 6.7 Hz, 2H), 2.19 – 2.12 (m, 1H), 2.11 (s, 3H), 1.99 – 1.89 (m, 1H), 1.82 (s, 3H), 1.80 (d, *J* = 7.3 Hz, 3H), 1.60 (dd, *J* = 5.9, 3.5 Hz, 1H), 1.54 – 1.38 (m, 5H), 1.17 (d, *J* = 6.3 Hz, 3H), 1.13 (d, *J* = 6.1 Hz, 3H).

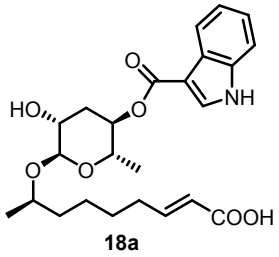
^{13}C NMR (101 MHz, $CDCl_3$) δ 170.4, 167.1, 167.1, 149.4, 137.8, 128.4, 121.0, 93.7, 72.2, 70.6, 69.8, 67.0, 51.4, 36.8, 32.2, 29.5, 27.9, 25.2, 21.3, 19.0, 17.7, 14.4, 12.1.

HR-ESI-MS (m/z) calcd for $C_{23}H_{36}NaO_8^+$ $[M+Na]^+$ 463.2302, found 462.2295.

The procedure for preparation of 18a-18b (exemplified by 18a).



To a solution of **17a** (45 mg, 0.09 mmol) in 1,4-dioxane (2 mL) was added aqueous LiOH (6.4 mg，0.27mmol) in 0.2 mL H_2O. The resulting mixture was stirred at 40 °C for 3 h. The aqueous layer was acidified to *p*H 2 with aqueous HCl solution (1.0 mol/L), and was extracted with Et_2O (10 mL × 3). The organic layers were washed with brine (10 mL × 3), dried over anhydrous Na_2SO_4, and concentrated in vacuum. The crude product was purified by silica gel column chromatography (DCM : MeOH = 10 : 1) to afford **18a** (32 mg, 0.07 mmol, 80%) as yellow oil.



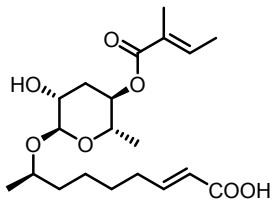
(R,E)-8-(((2R,3R,5R,6S)-5-((1H-indole-3-carbonyl)oxy)-3-hydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)non-2-enoic acid (18a).

The spectral data of **18a** are identical to those of a previous report.[7]

^{1}H NMR (400 MHz, CD_3OD) δ 8.04 (dd, *J* = 6.6, 1.5 Hz, 1H), 8.00 (s, 1H), 7.47 (dd, *J* = 6.7, 1.6 Hz, 1H), 7.25 – 7.17 (m, 2H), 7.05 – 6.94 (m, 1H), 5.83 (d, *J* = 15.6 Hz, 1H), 5.15 (td, *J* = 10.4, 4.7 Hz, 1H), 4.77 (s, 1H), 4.07 (dq, *J* = 9.8, 6.2 Hz, 1H), 3.89 – 3.80 (m, 2H), 2.34 – 2.20 (m, 3H), 2.08 – 1.97 (m, 1H), 1.67 – 1.46 (m, 6H), 1.26 (d, *J* = 6.3 Hz, 3H), 1.17 (d, *J* = 6.0 Hz, 3H).

^{13}C NMR (101 MHz, CD_3OD) δ 168.8, 165.0, 149.7, 136.8, 132.1, 125.9, 122.4, 121.3, 121.3, 120.5, 111.7, 106.9, 96.2, 71.3, 69.2, 68.2, 67.4, 36.7, 32.1, 31.9, 27.8, 25.3, 18.0, 16.9.

HR-ESI-MS (m/z) calcd for $C_{24}H_{31}NNaO_7^+$ $[M+Na]^+$ 468.1993, found 468.1992.



18b

(R,E)-8-(((2R,3R,5R,6S)-3-hydroxy-6-methyl-5-(((E)-2-methylbut-2-enoyl)oxy)tetrahydro-2H-pyran-2-yl)oxy)non-2-enoic acid (18b).

18b (17 mg, 0.044 mmol, 53%) was obtained from **17b** (37 mg, 0.084 mmol) as yellow oil.

The spectral data of **18b** are identical to those of a previous report.[6]

^{1}H NMR (400 MHz, CD_3OD) δ 6.98 (dt, *J* = 15.2, 7.0 Hz, 1H), 6.91 – 6.83 (m, 1H), 5.83 (d, *J* = 15.6 Hz, 1H), 4.91 – 4.88 (m, 1H), 4.72 (s, 1H), 3.95 – 3.86 (m, 1H), 3.86 – 3.78 (m, 1H), 3.75 (d, *J* = 0.8 Hz, 1H), 2.28 (dd, *J* = 12.7, 6.4 Hz, 2H), 2.09 (dt, *J* = 12.8, 3.8 Hz, 1H), 1.91 – 1.85 (m, 1H), 1.83 (d, *J* = 6.4 Hz, 6H), 1.67 – 1.42 (m, 6H), 1.16 (dd, *J* = 5.9, 4.8 Hz, 6H).

^{13}C NMR (101 MHz, CD_3OD) δ 168.6, 167.2, 149.6, 137.6, 128.2, 121.3, 96.2, 71.3, 70.0, 68.1, 67.0, 36.6, 31.8, 31.6, 27.7, 25.1, 17.9, 16.7, 13.0, 10.7.

HR-ESI-MS (m/z) calcd for $C_{20}H_{32}NaO_7^+$ $[M+Na]^+$ 407.2040, found 407.2043.

ROS assay. The roots of 10-day-old *Arabidopsis thaliana* Col-0 grown on half-strength MS plates were cut and floated in a 96-well plate containing 100 μL ddH_2O for 12 h. Afterwards, the ddH_2O was replaced with 100 μL reaction solution containing 3 μg/mL horseradish peroxidase, 2.5 μM 8-amino-5-chloro-2,3-dihydro-7-phenyl-pyrido[3,4-d] pyridazine sodium salt (L-012, Wako Chemical) with 1 μM ascarosides supplementation**.** And ddH_2O and 50 nM flg22 were used as negative and positive controls. Measurement was conducted with a luminometer immediately after adding the reaction solution. ROS production values from eight roots per treatment are expressed as the mean relative light units. The experiment was repeated three times with similar results.

Figure S1. The ^{1}H NMR spectra copy of compound 2 in $CDCl_3$ (500 MHz)

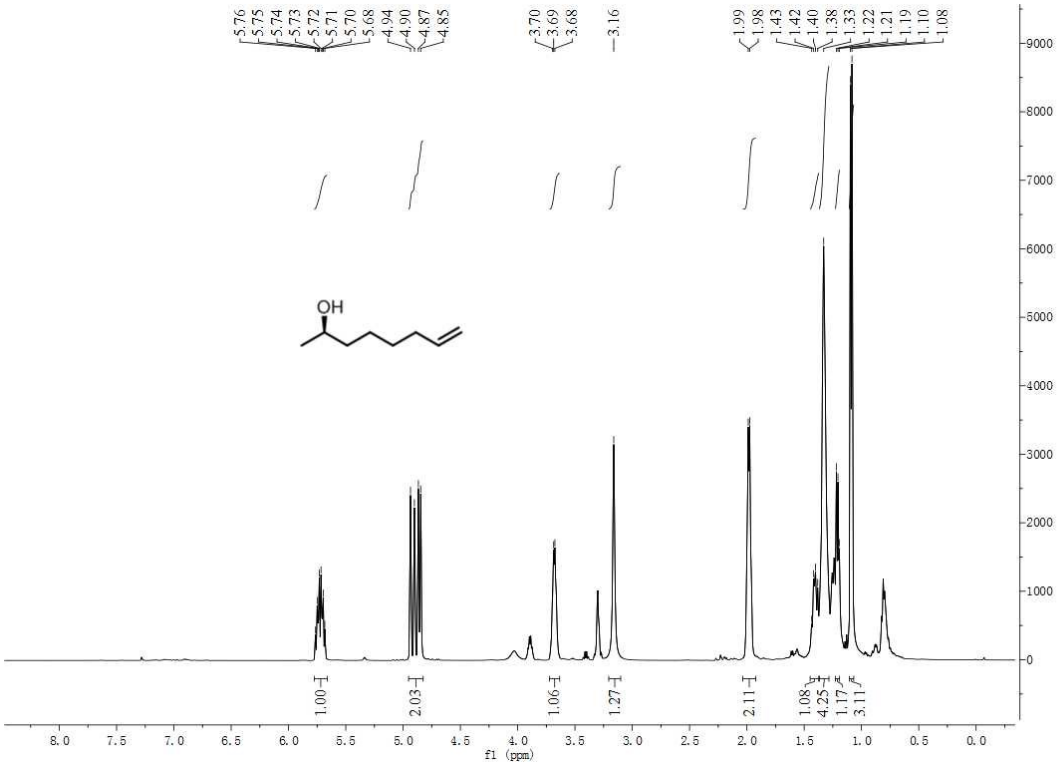


Figure S2. The ^{13}C NMR spectra copy of compound 2 $CDCl_3$ (126 MHz)

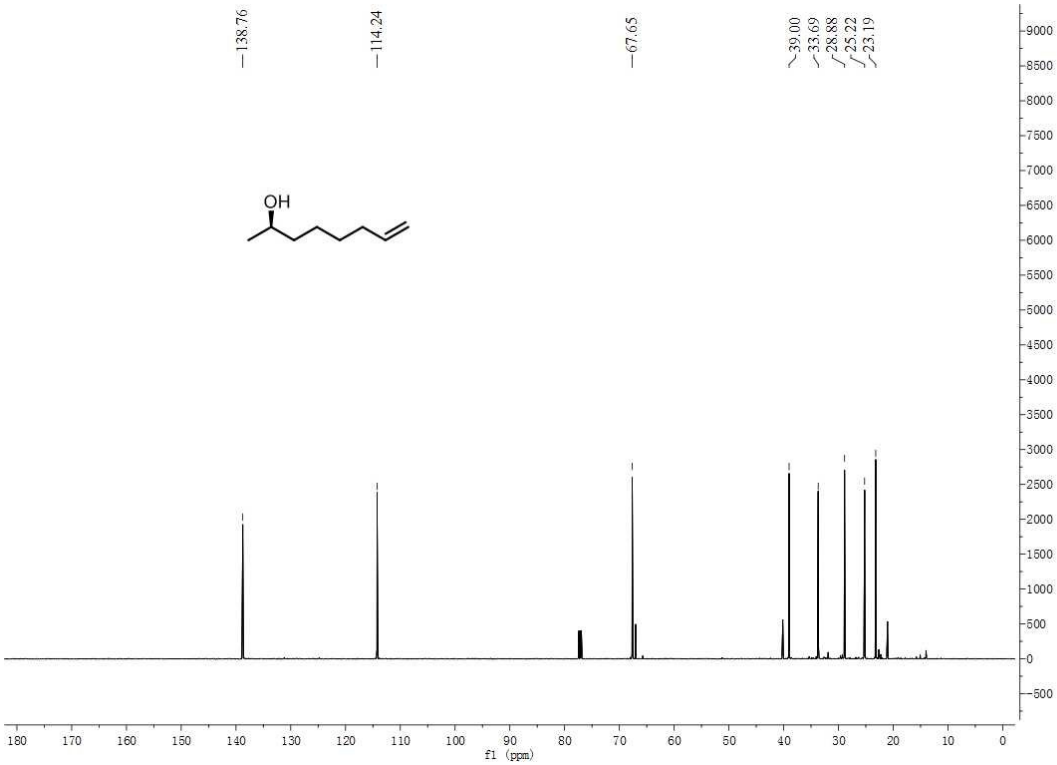


Figure S3. The ^{1}H NMR spectra copy of compound 3-1 in $CDCl_3$ (400 MHz)

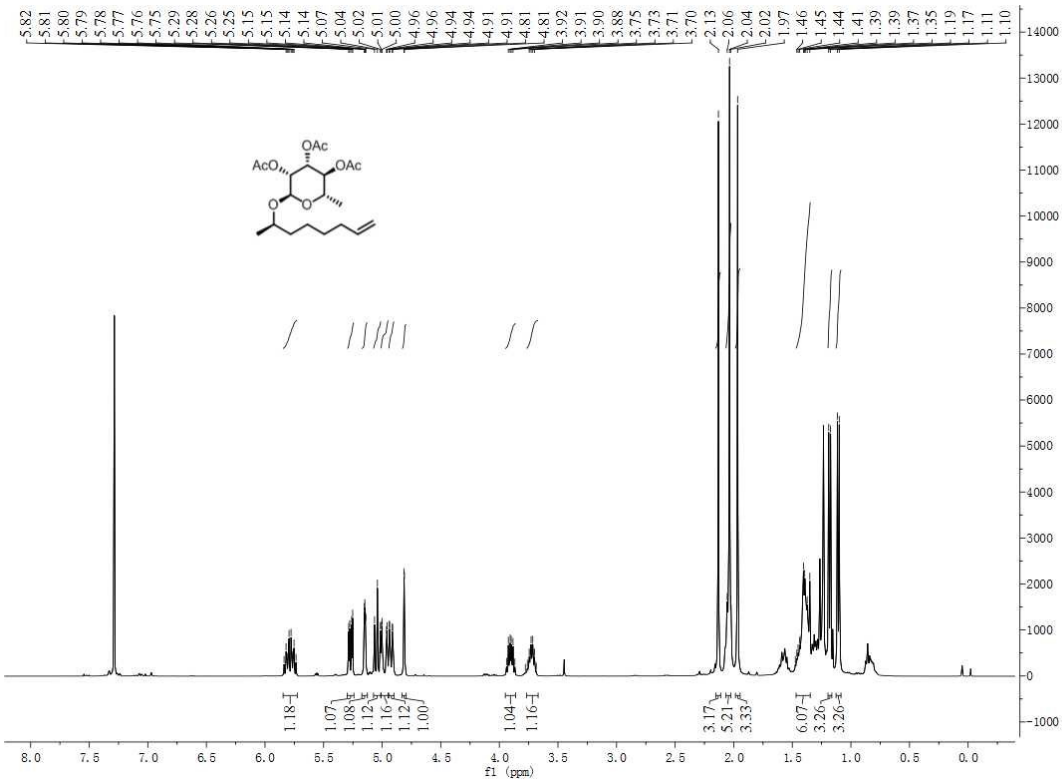


Figure S4. The ^{13}C NMR spectra copy of compound 3-1 in $CDCl_3$ (101 MHz)

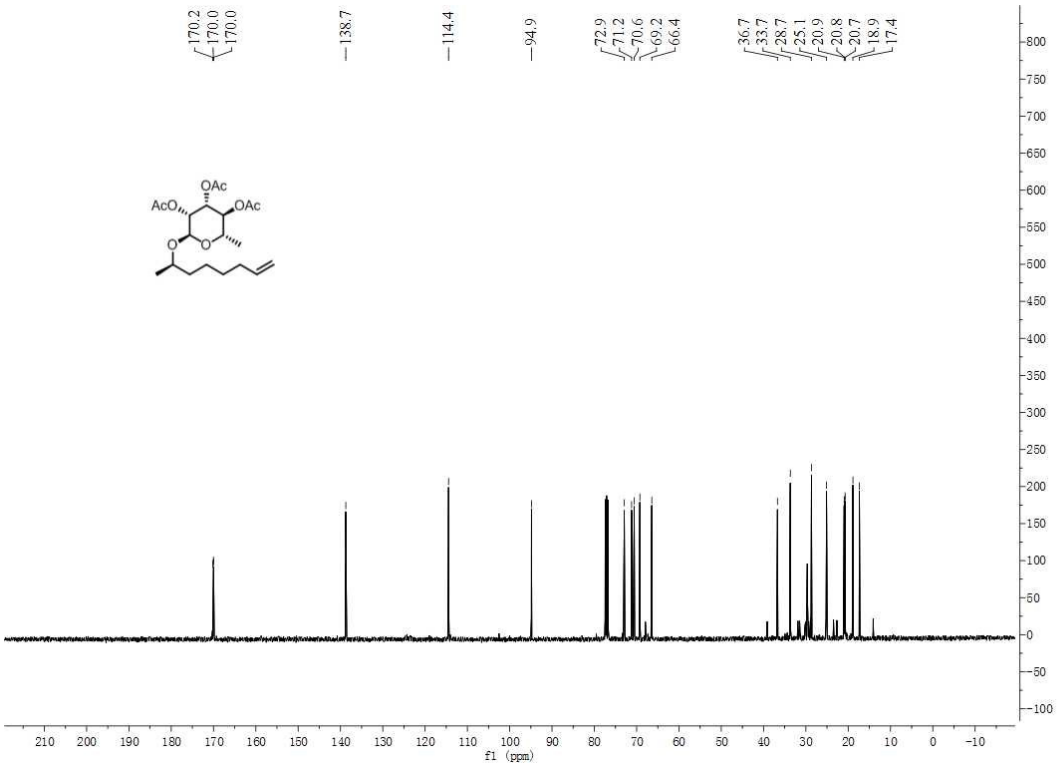


Figure S5. The ^{1}H NMR spectra copy of compound 3 in $CDCl_3$ (400 MHz)

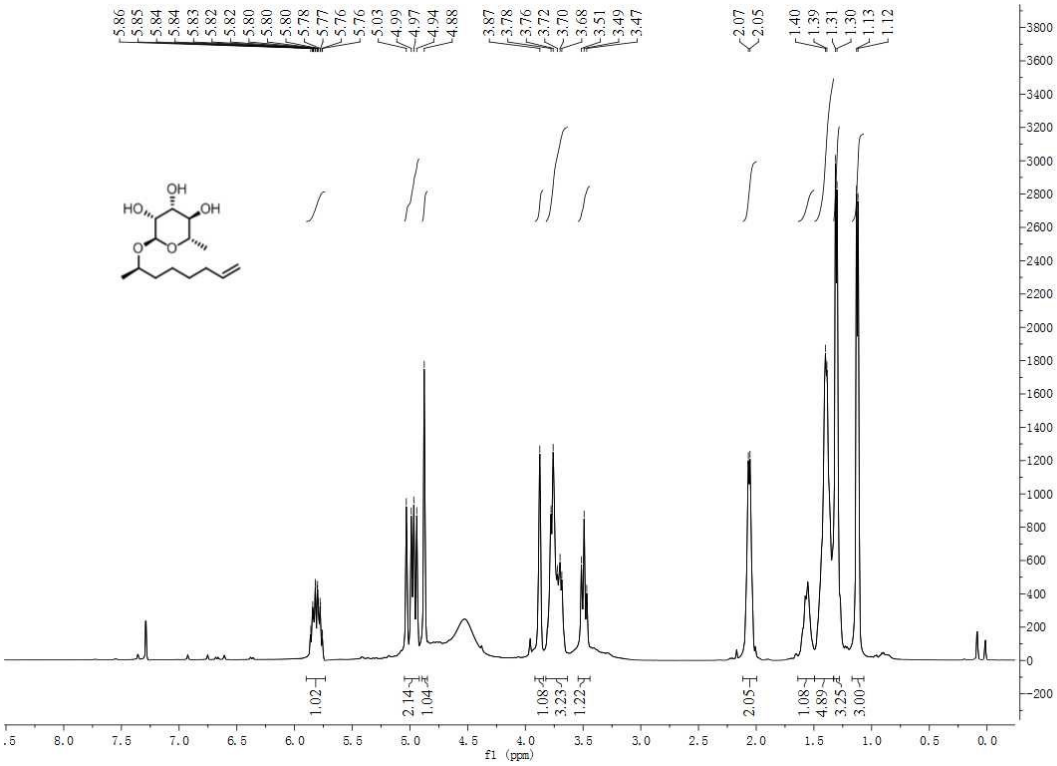


Figure S6. The ^{13}C NMR spectra copy of compound 3 in $CDCl_3$ (101 MHz)

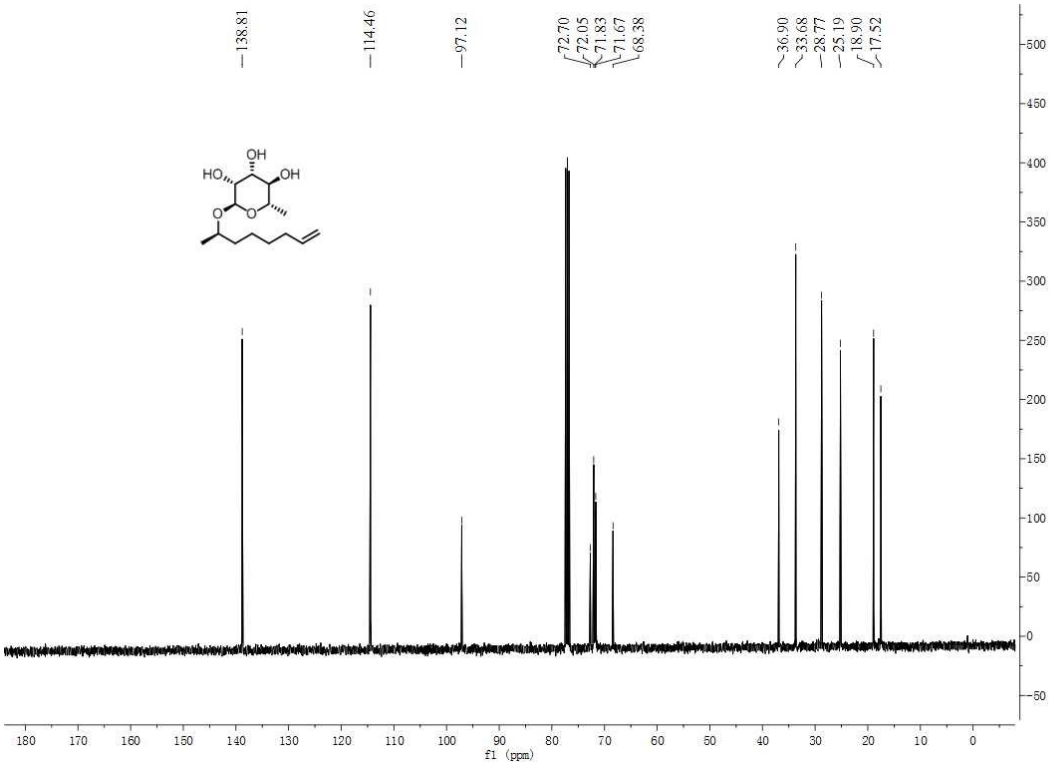


Figure S7. The ^{1}H NMR spectra copy of compound 4 in $CDCl_3$ (400 MHz)

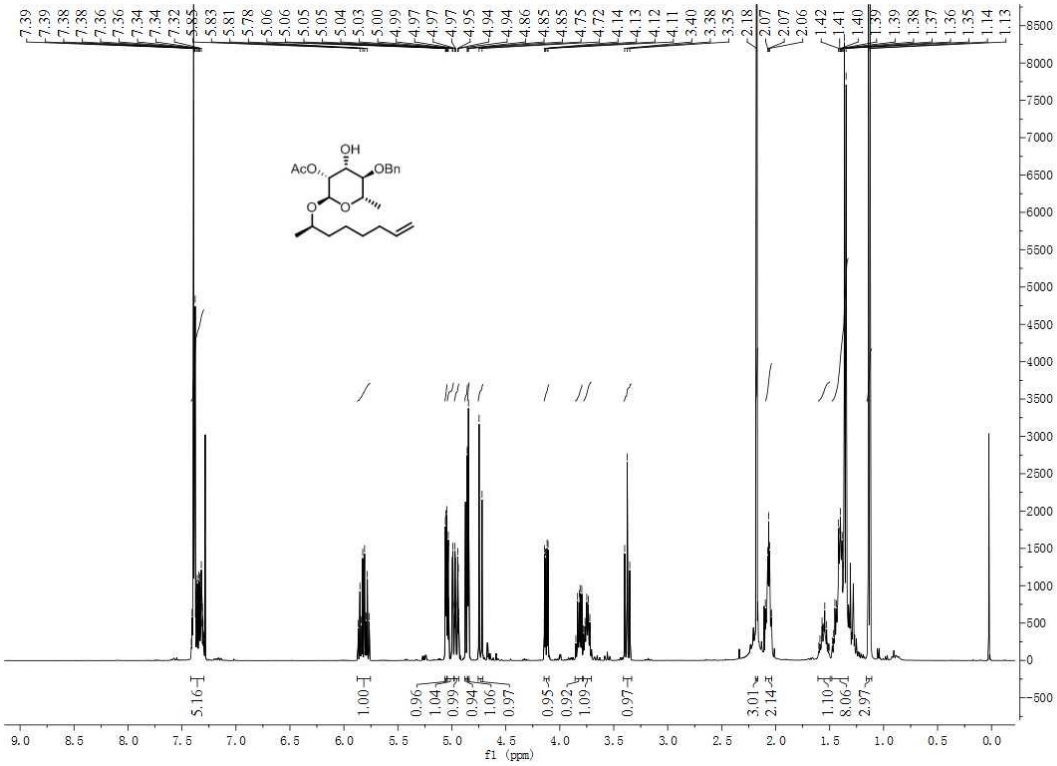


Figure S8. The ^{13}C NMR spectra copy of compound 4 in $CDCl_3$ (101 MHz)

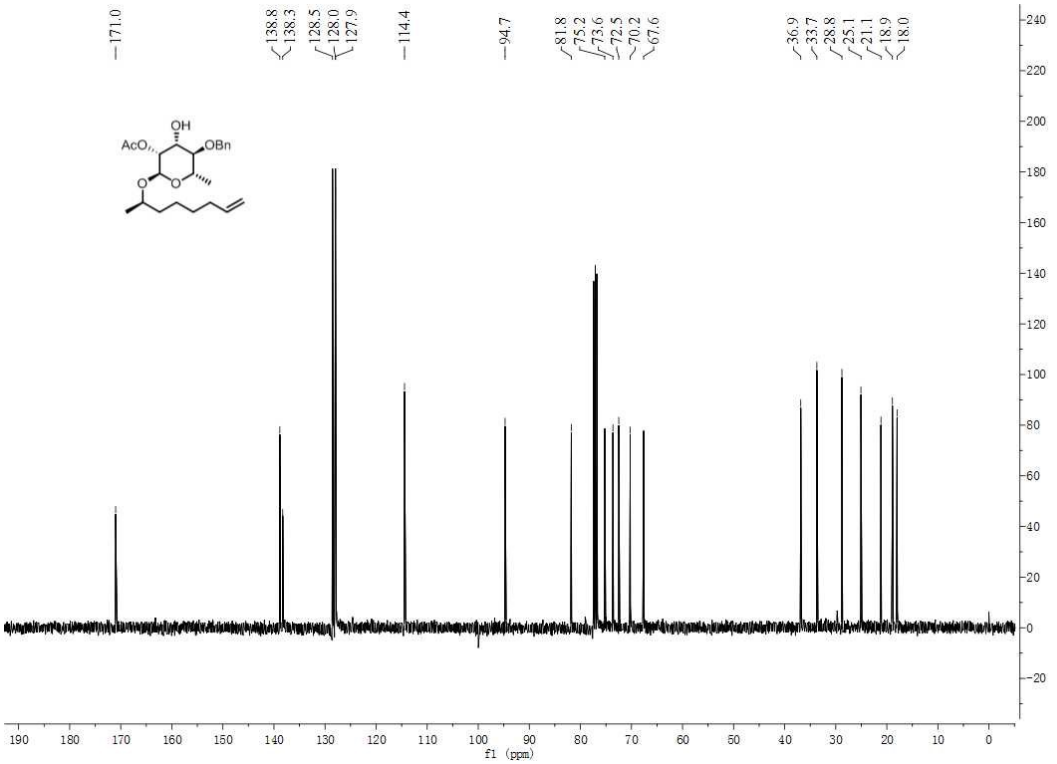


Figure S9. The ^{1}H NMR spectra copy of compound 5 in $CDCl_3$ (400 MHz)

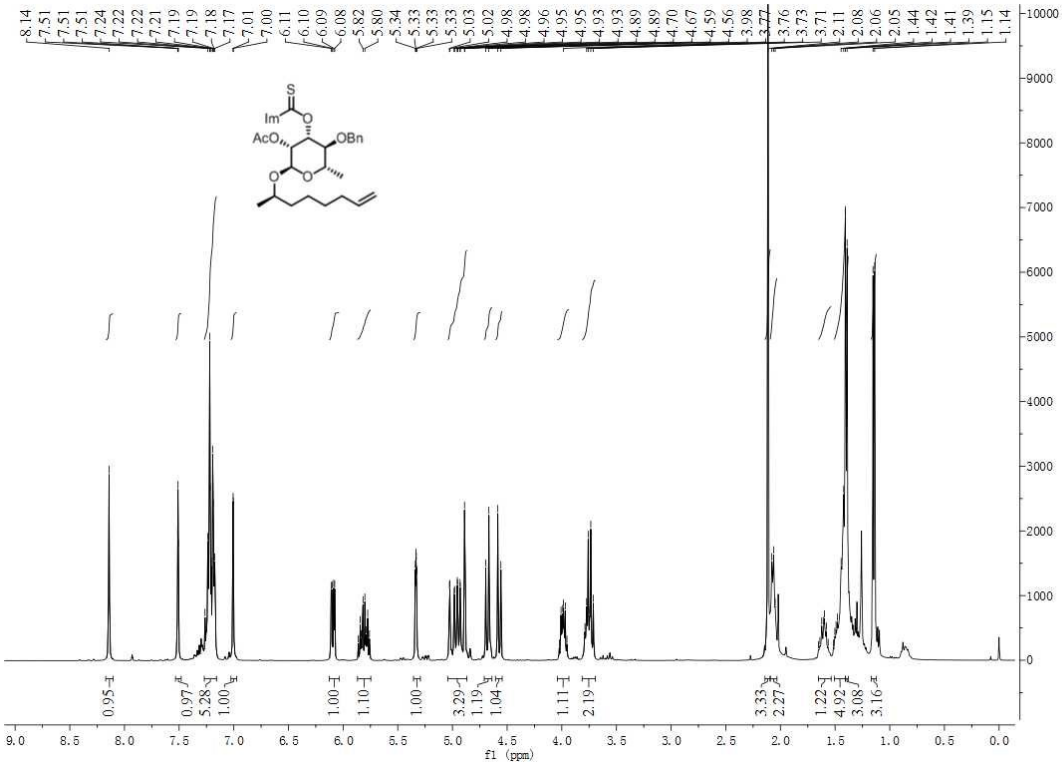


Figure S10. The ^{13}C NMR spectra copy of compound 5 in $CDCl_3$ (101 MHz)

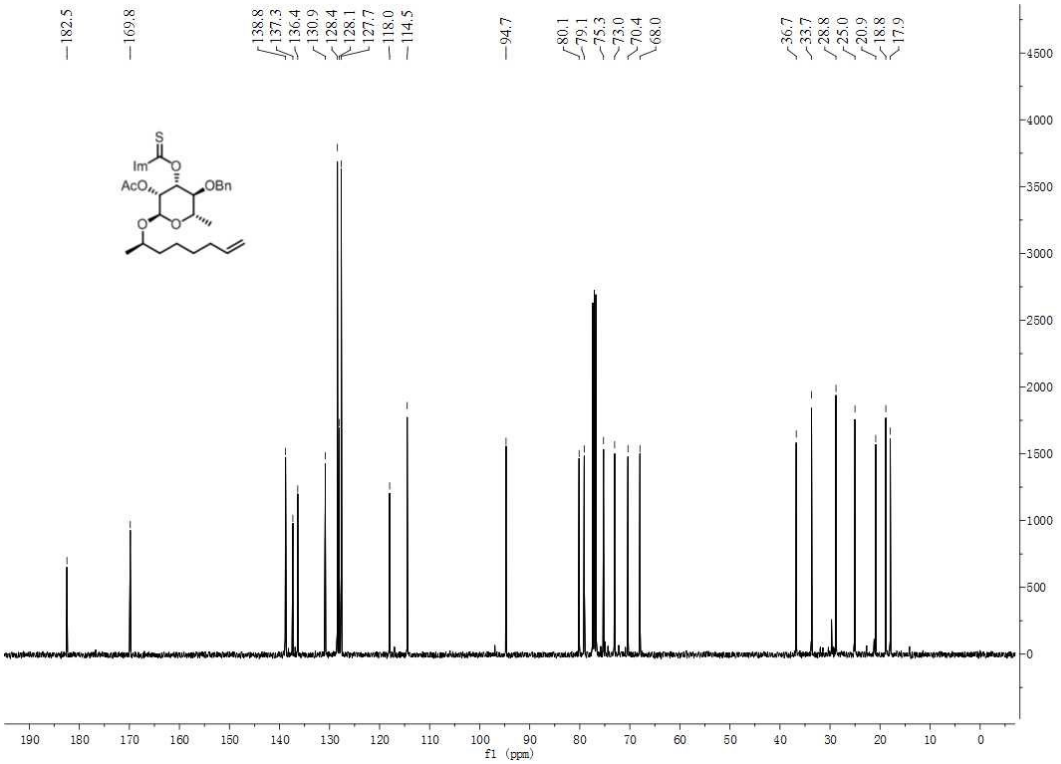


Figure S11. The ^{1}H NMR spectra copy of compound 6 in $CDCl_3$ (400 MHz)

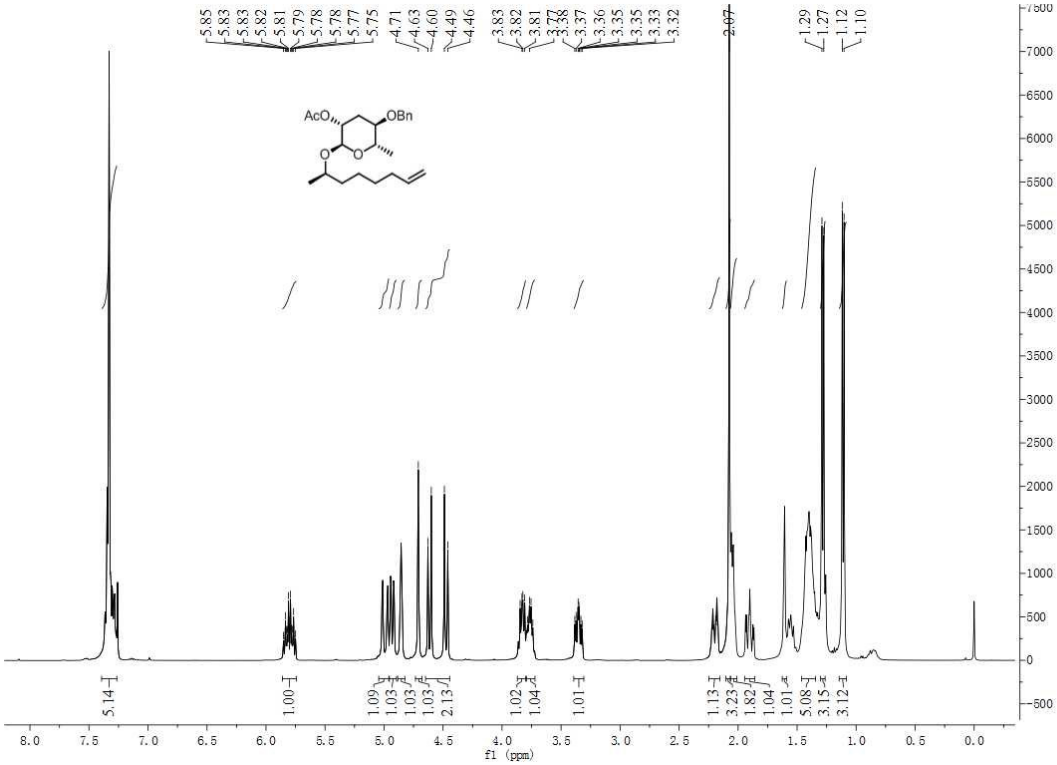


Figure S12. The ^{13}C NMR spectra copy of compound 6 in $CDCl_3$ (101 MHz)

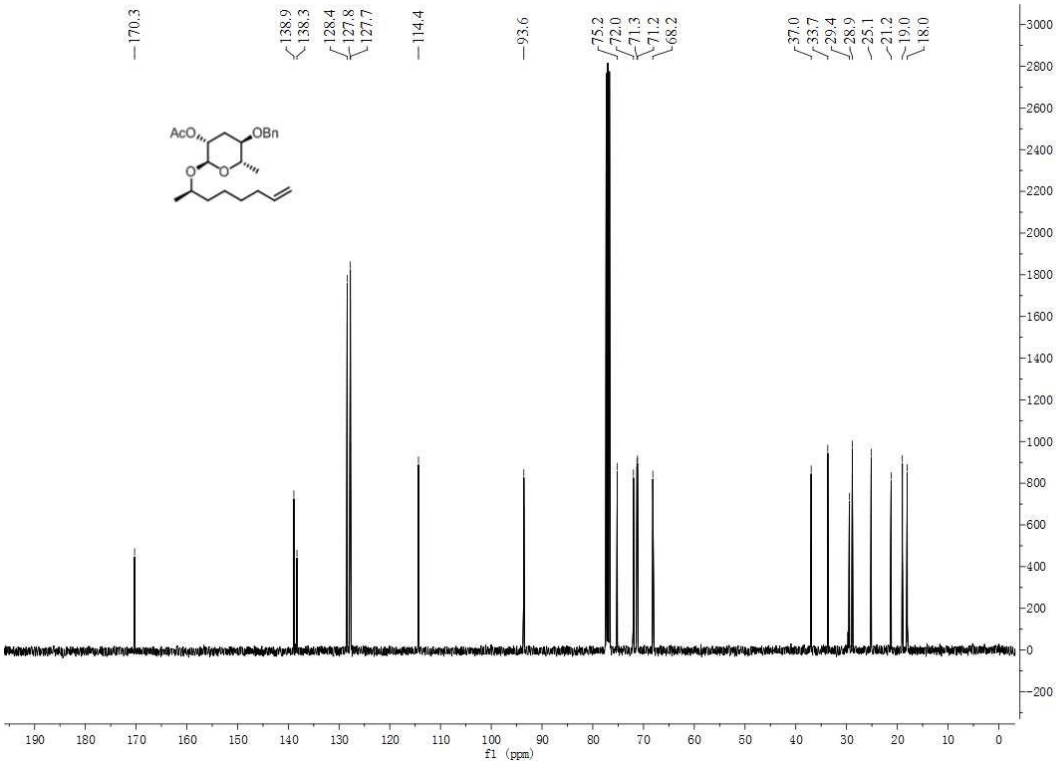


Figure S13 The ^{1}H NMR spectra copy of the inseparable mixture 7-1 in $CDCl_3$ (400 MHz)

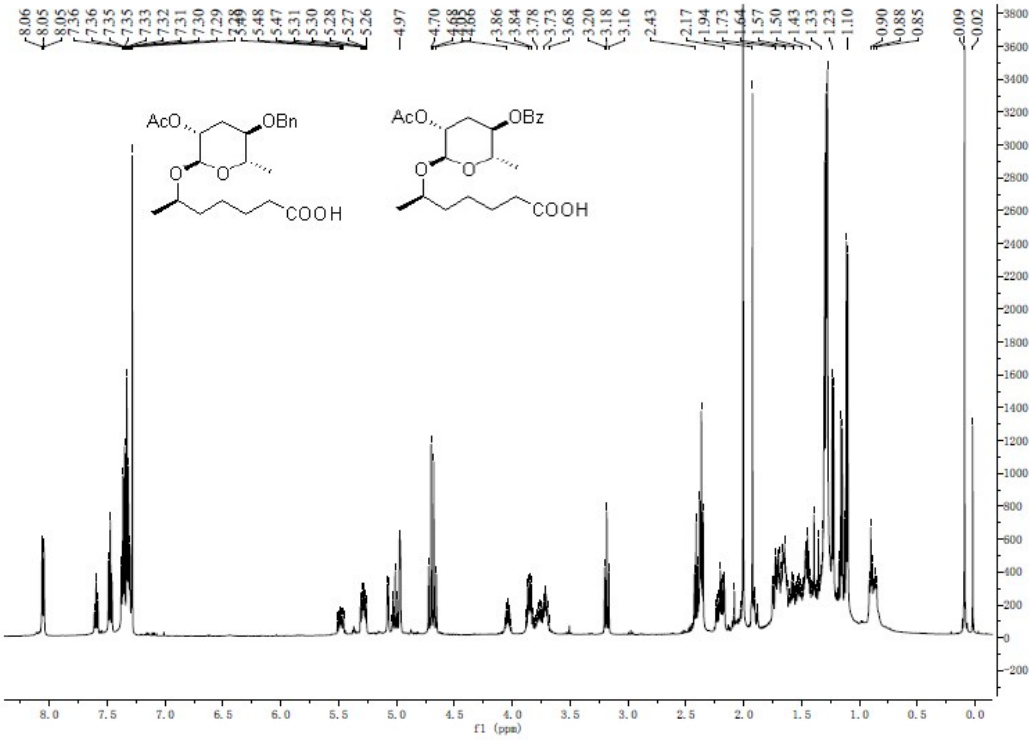


Figure S14. The ^{1}H NMR spectra copy of compound 8-1 in $CDCl_3$ (400 MHz)

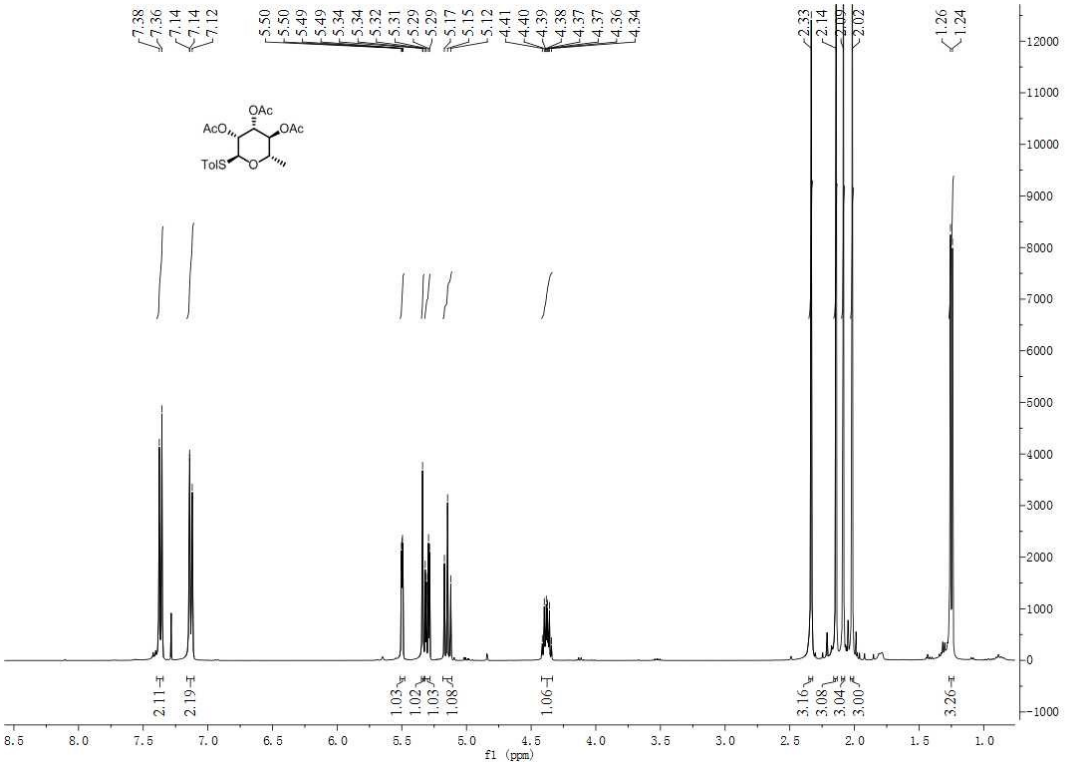


Figure S15. The ^{13}C NMR spectra copy of compound 8-1 in $CDCl_3$ (101 MHz)

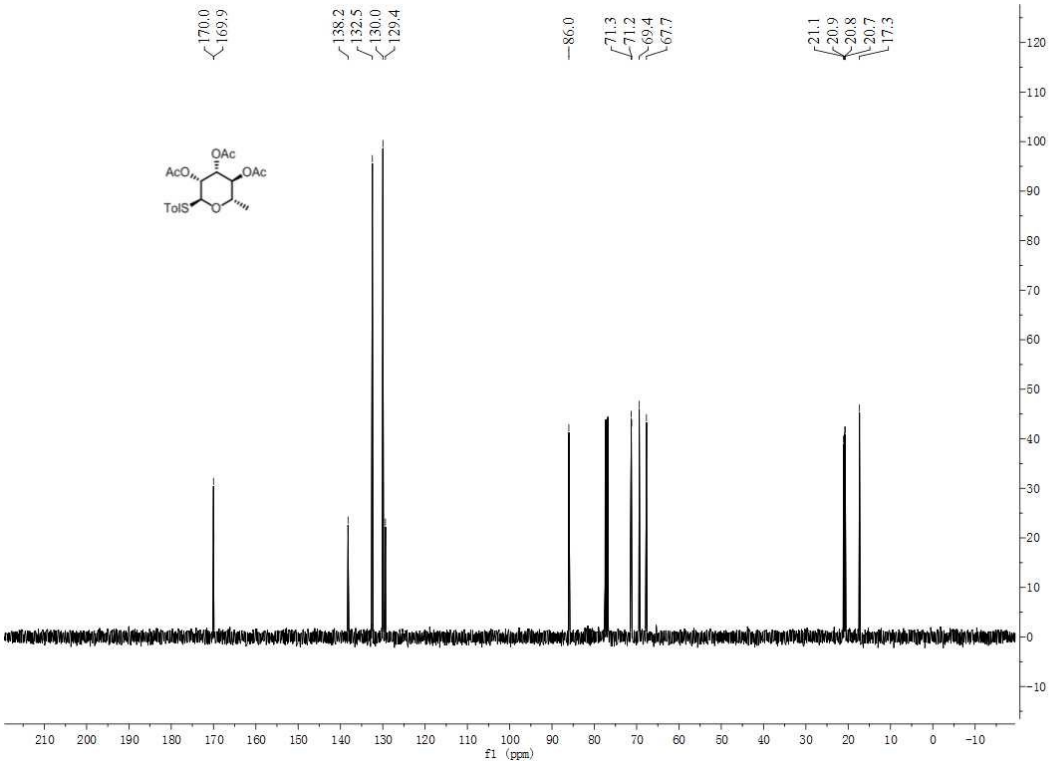


Figure S16. The ^{1}H NMR spectra copy of compound 8 in CD_3OD (400 MHz)

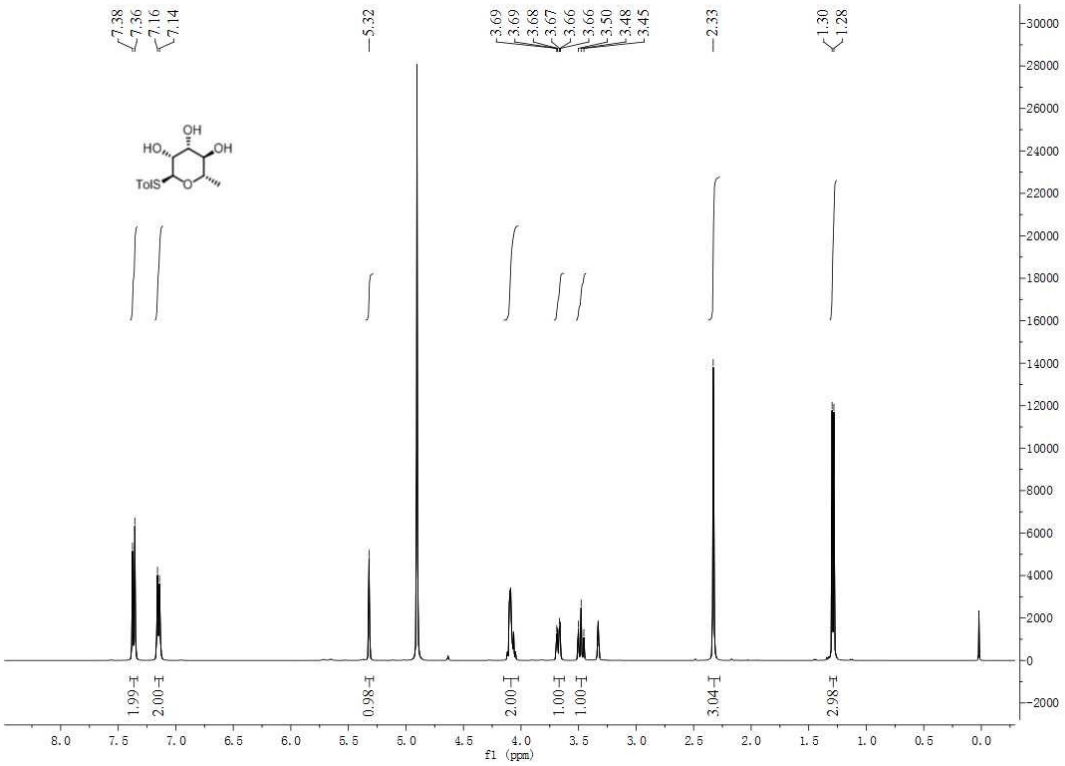


Figure S17. The ^{13}C NMR spectra copy of compound 8 in CD_3OD (101 MHz)

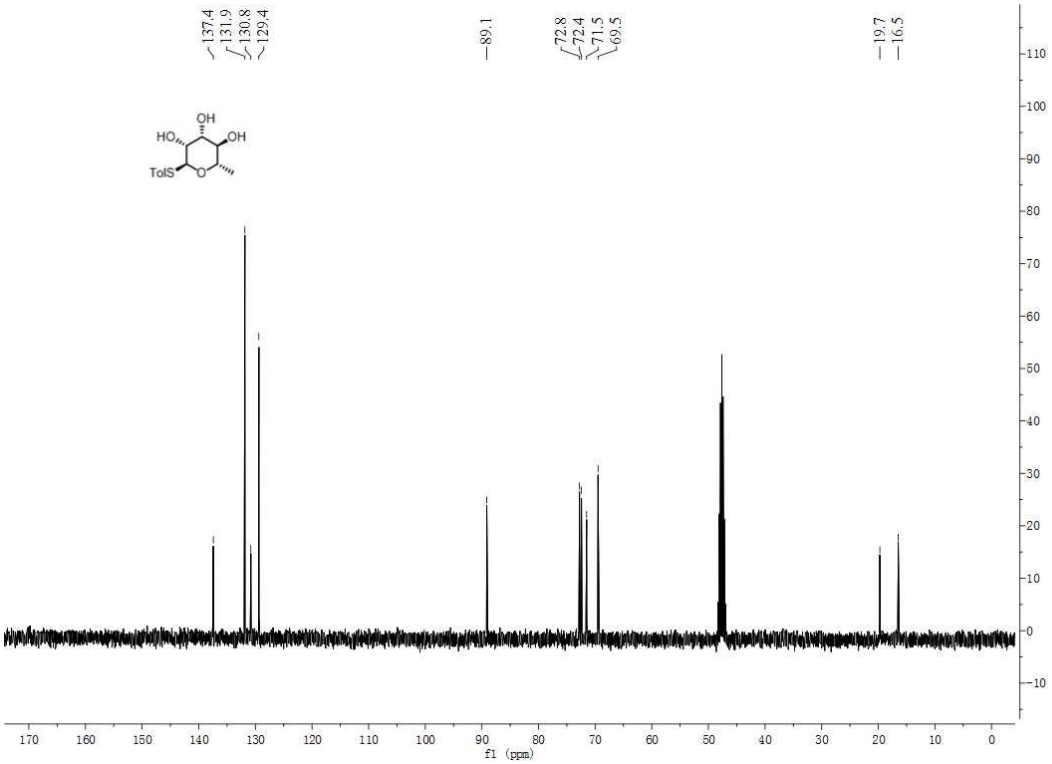


Figure S18. The ^{1}H NMR spectra copy of compound 9 in $CDCl_3$ (500 MHz)

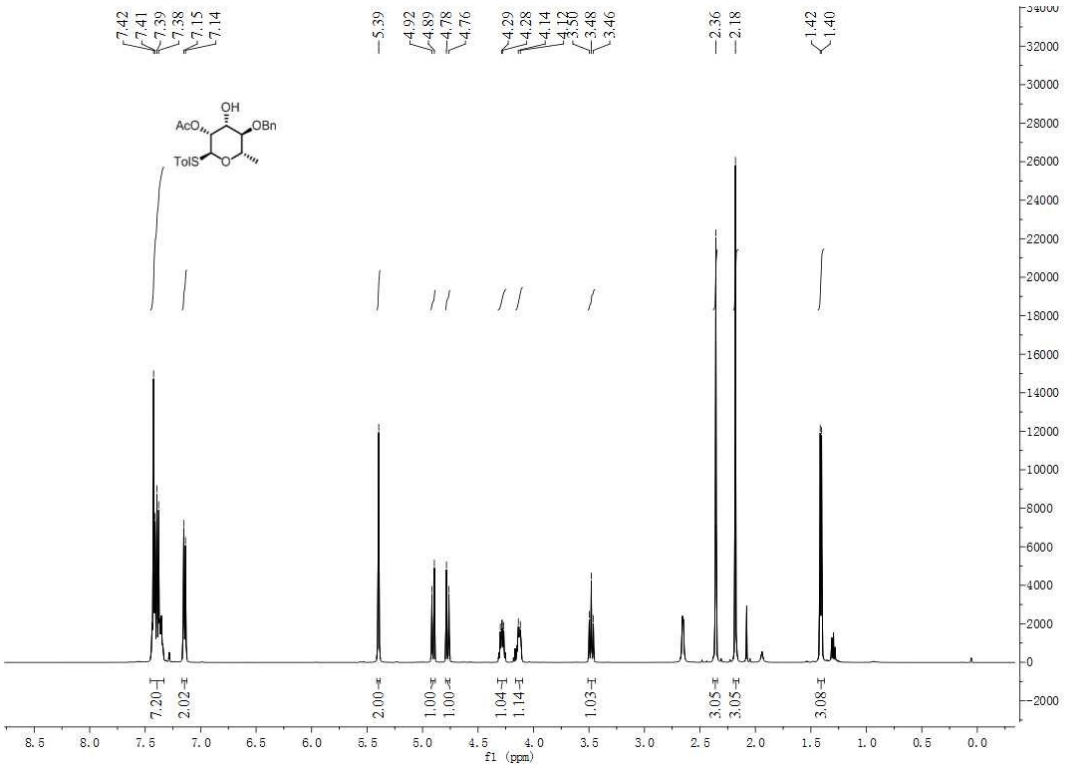


Figure S19. The ^{13}C NMR spectra copy of compound 9 in $CDCl_3$ (126 MHz)

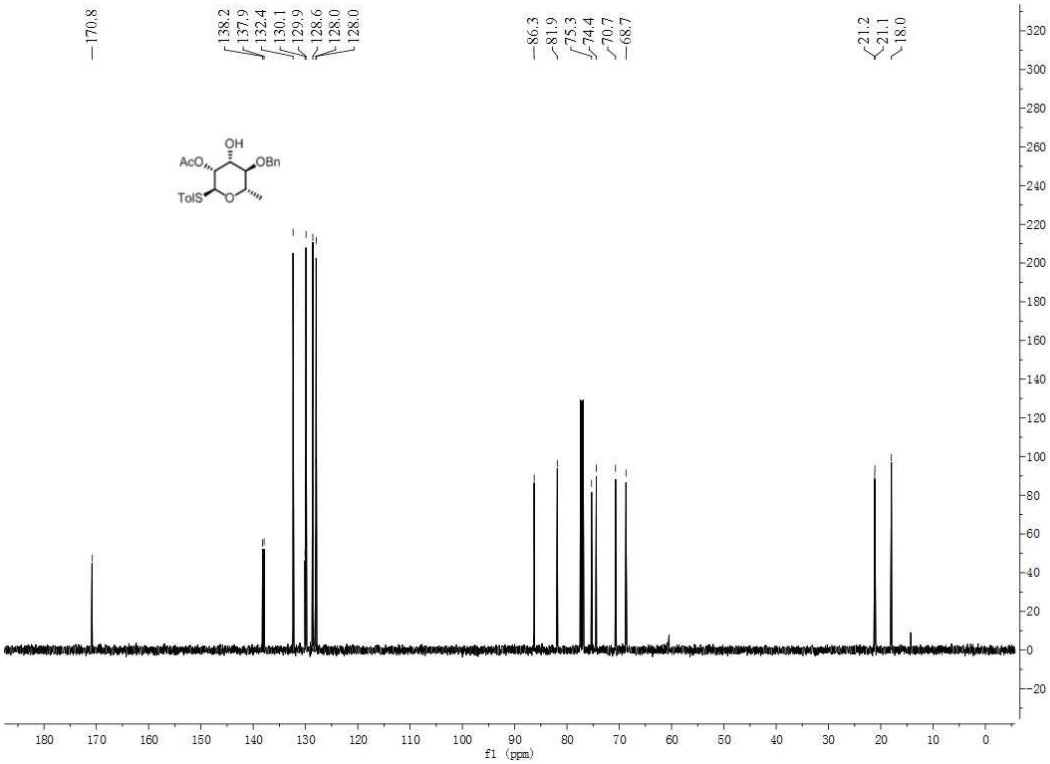


Figure S20. The ^{1}H NMR spectra copy of compound 10-1 in $CDCl_3$ (500 MHz)

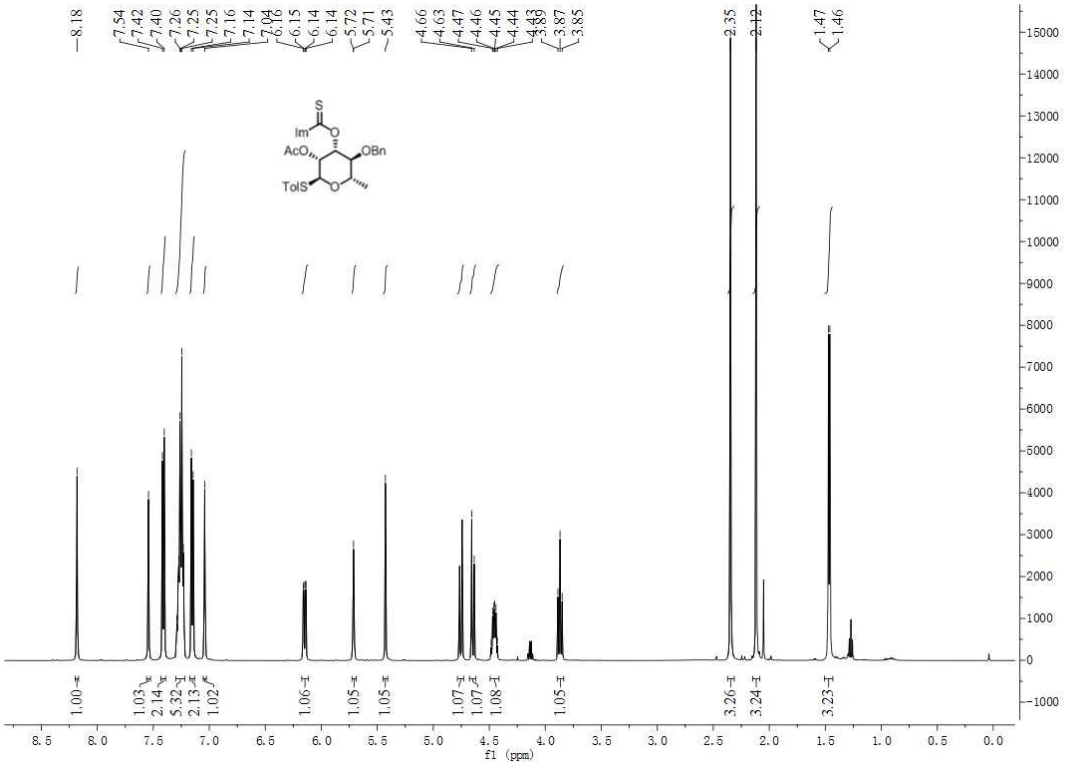


Figure S21. The ^{13}C NMR spectra copy of compound 10-1 in $CDCl_3$ (126 MHz)

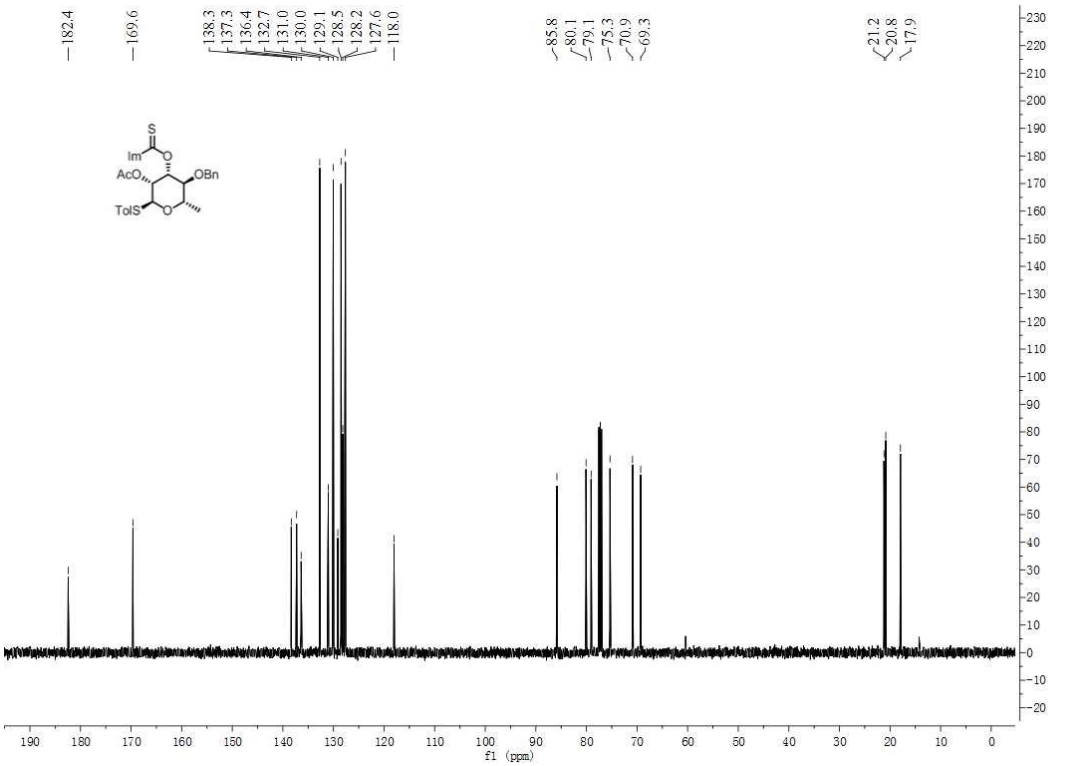


Figure S22. The ^{1}H NMR spectra copy of compound 10 in $CDCl_3$ (400 MHz)

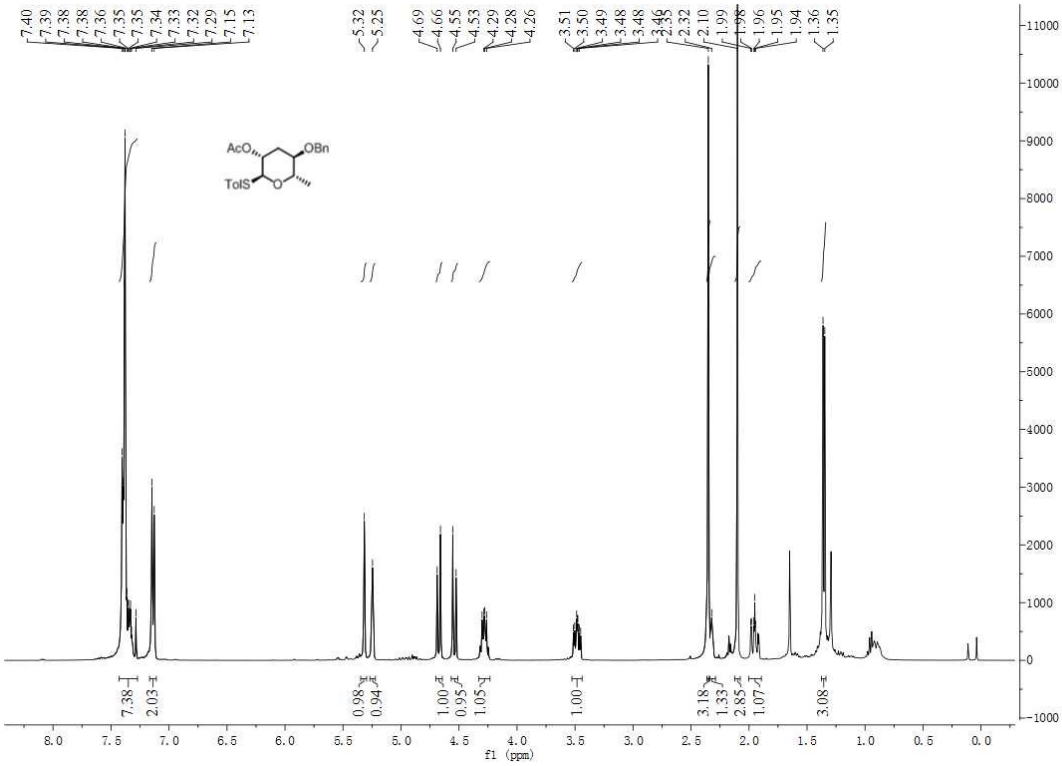


Figure S23. The ^{13}C NMR spectra copy of compound 10 in $CDCl_3$ (101 MHz)

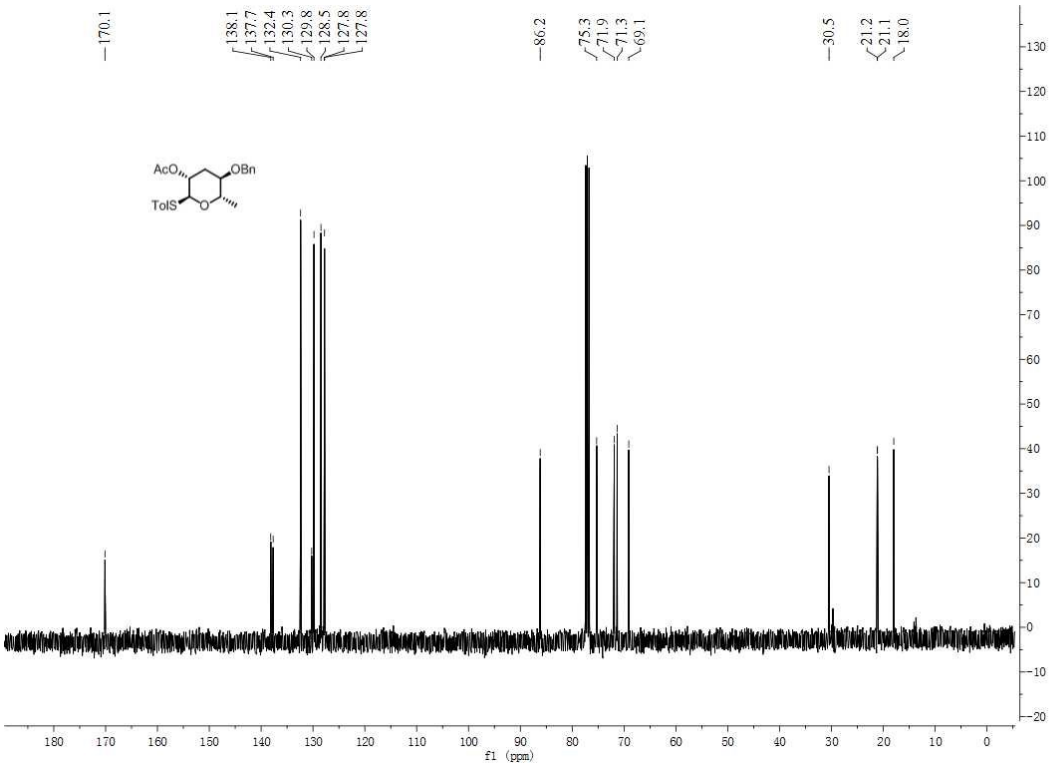


Figure S24. The ^{1}H NMR spectra copy of compound 11b-3 in $CDCl_3$ (101 MHz)

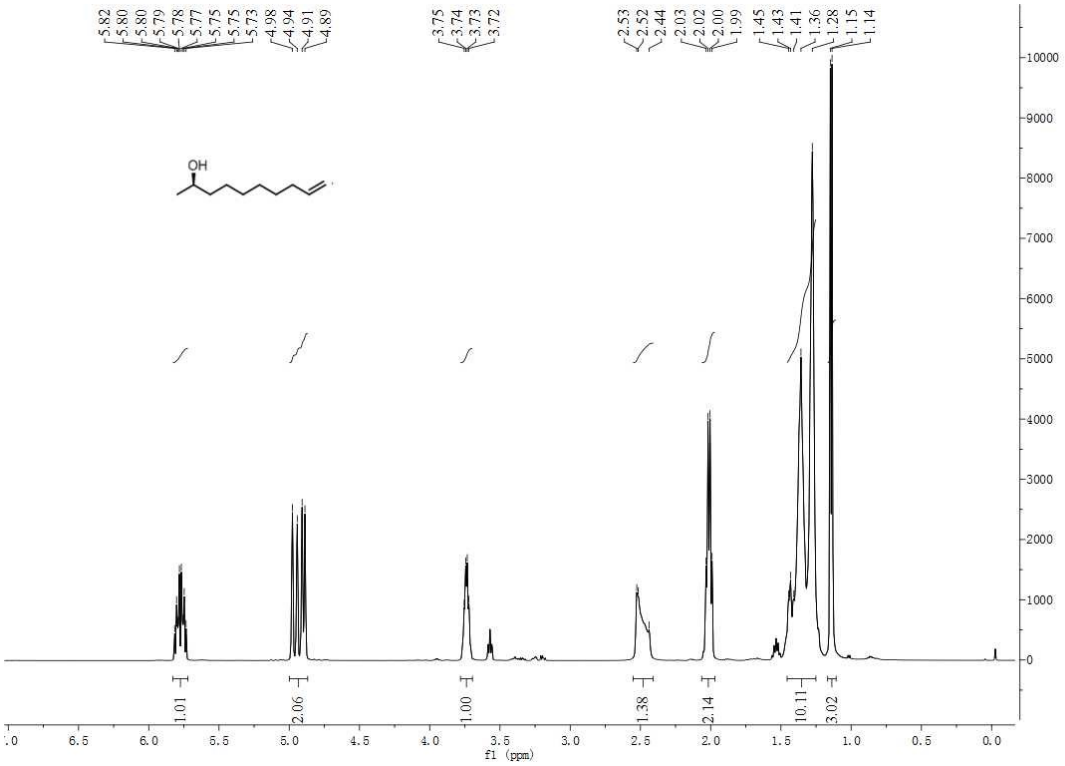


Figure S25. The ^{13}C NMR spectra copy of compound 11b-3 in $CDCl_3$ (126 MHz)

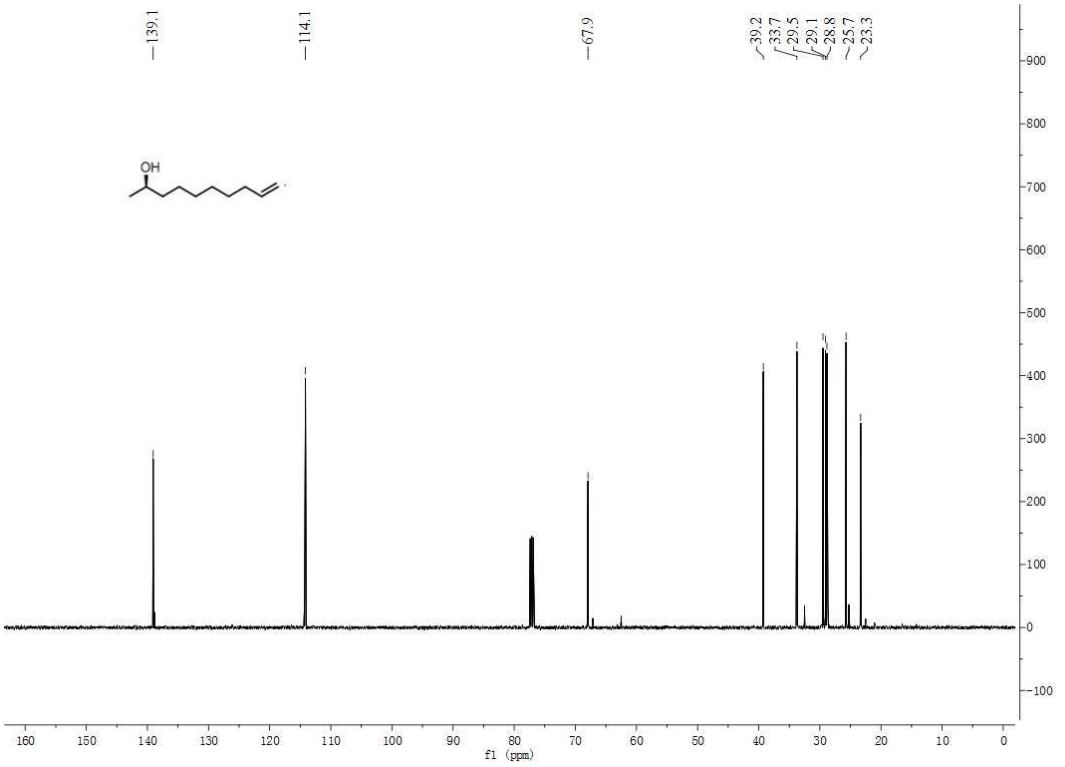


Figure S26. The ^{1}H NMR spectra copy of compound 11b-4 in $CDCl_3$ (400 MHz)

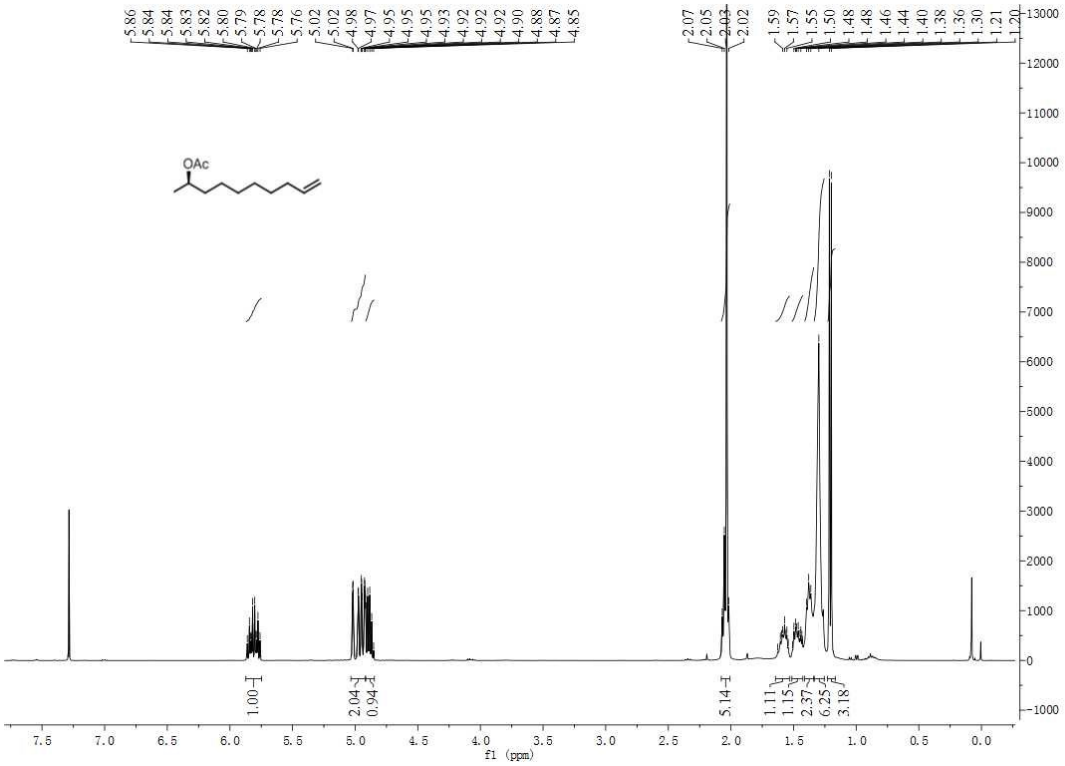


Figure S27. The ^{13}C NMR spectra copy of compound 11b-4 in $CDCl_3$ (101 MHz)

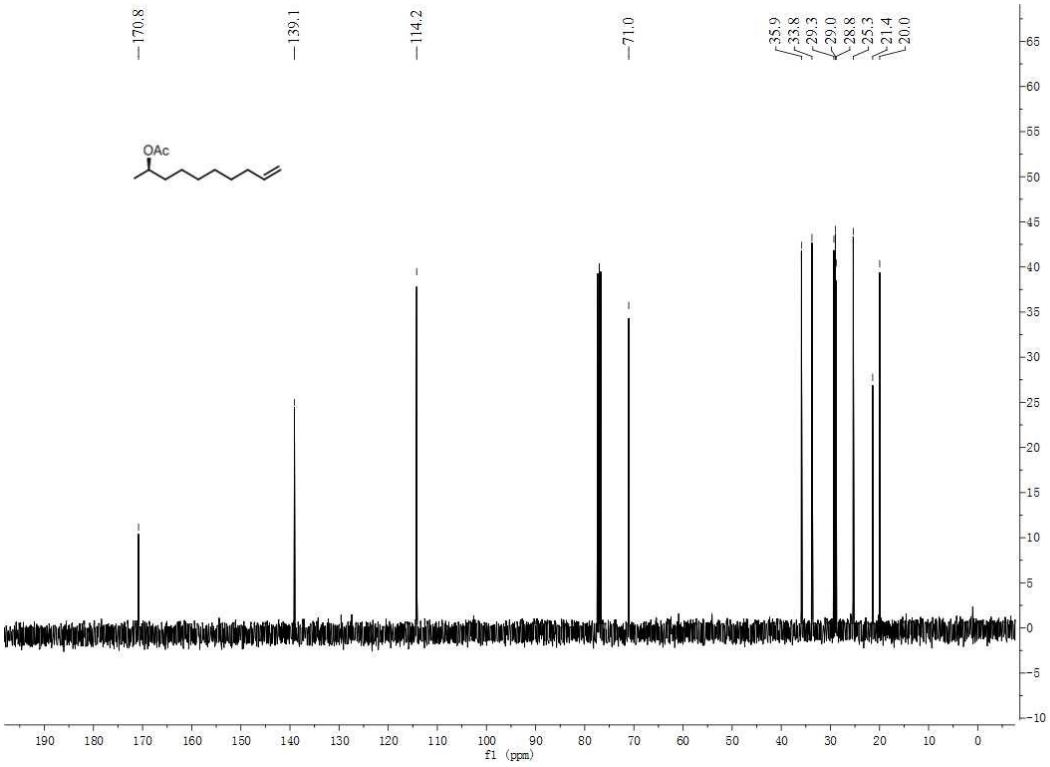


Figure S28. The ^{1}H NMR spectra copy of compound 11b-5 in $CDCl_3$ (400 MHz)

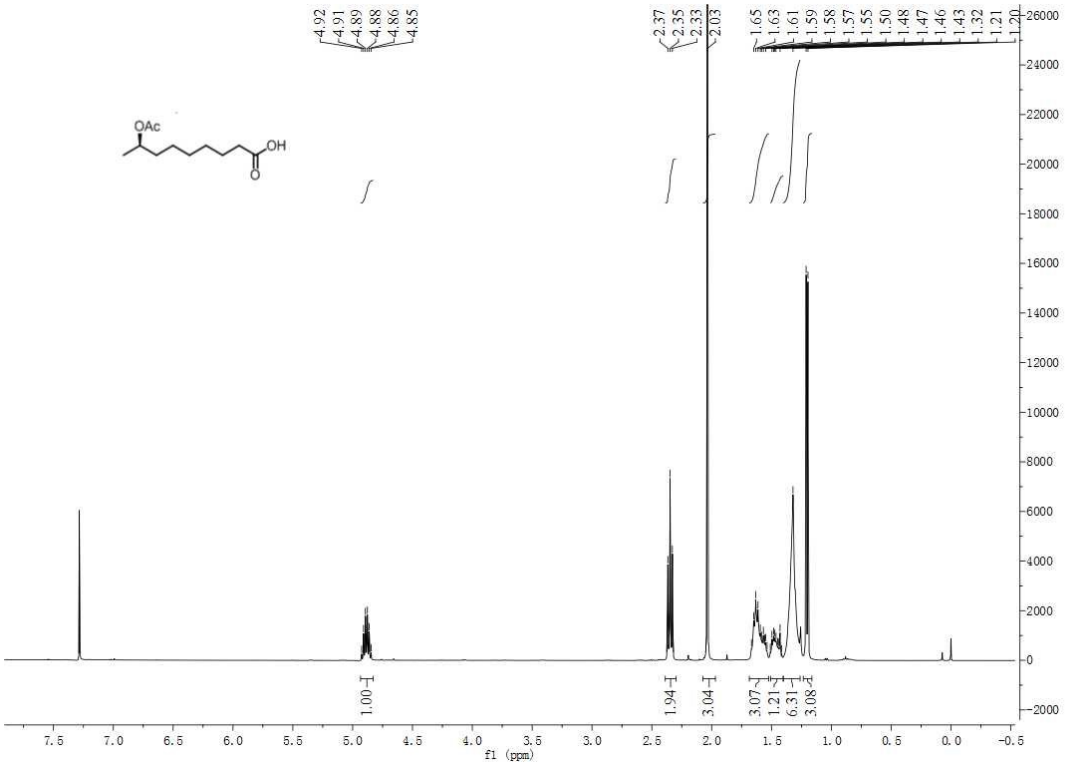


Figure S29. The ^{13}C NMR spectra copy of compound 11b-5 in $CDCl_3$ (101 MHz)

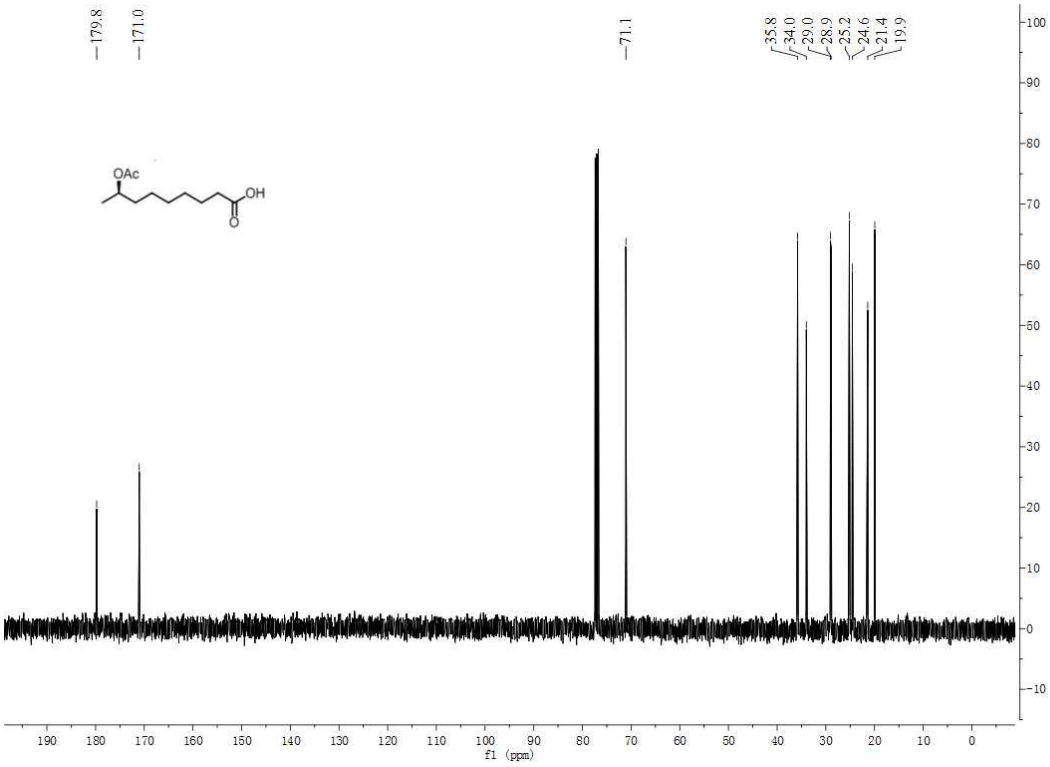


Figure S30. The ^{1}H NMR spectra copy of compound 11b-6 in $CDCl_3$ (400 MHz)

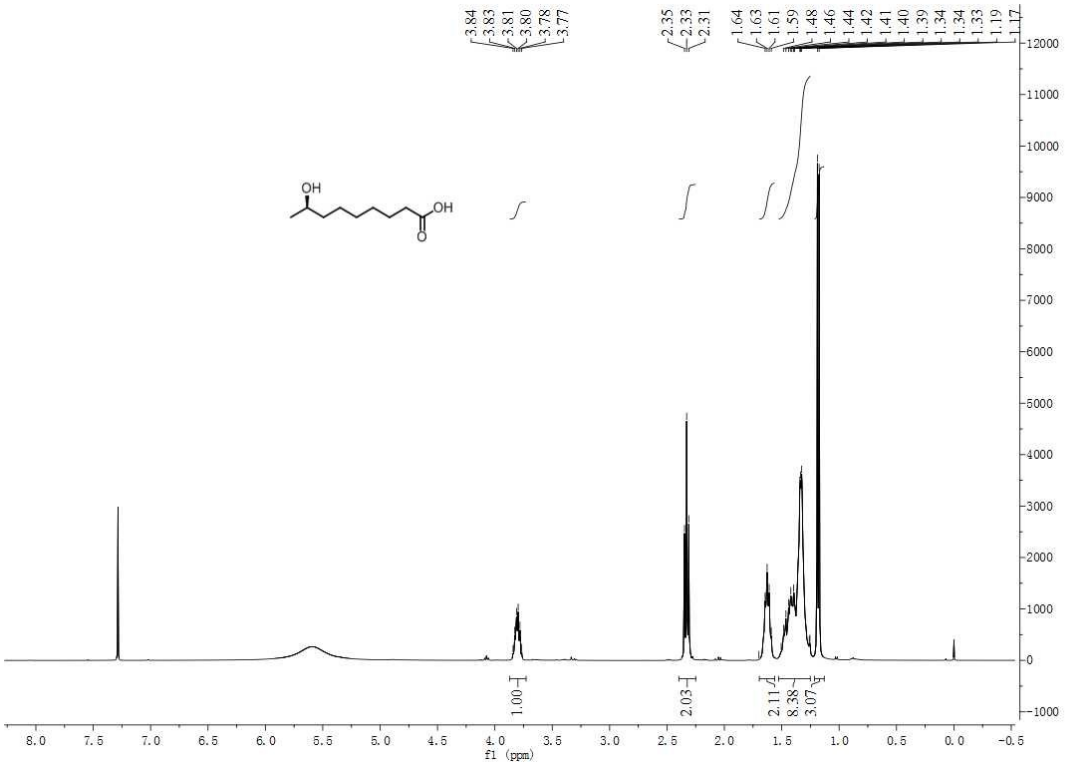


Figure S31. The ^{13}C NMR spectra copy of compound 11b-6 in $CDCl_3$ (101 MHz)

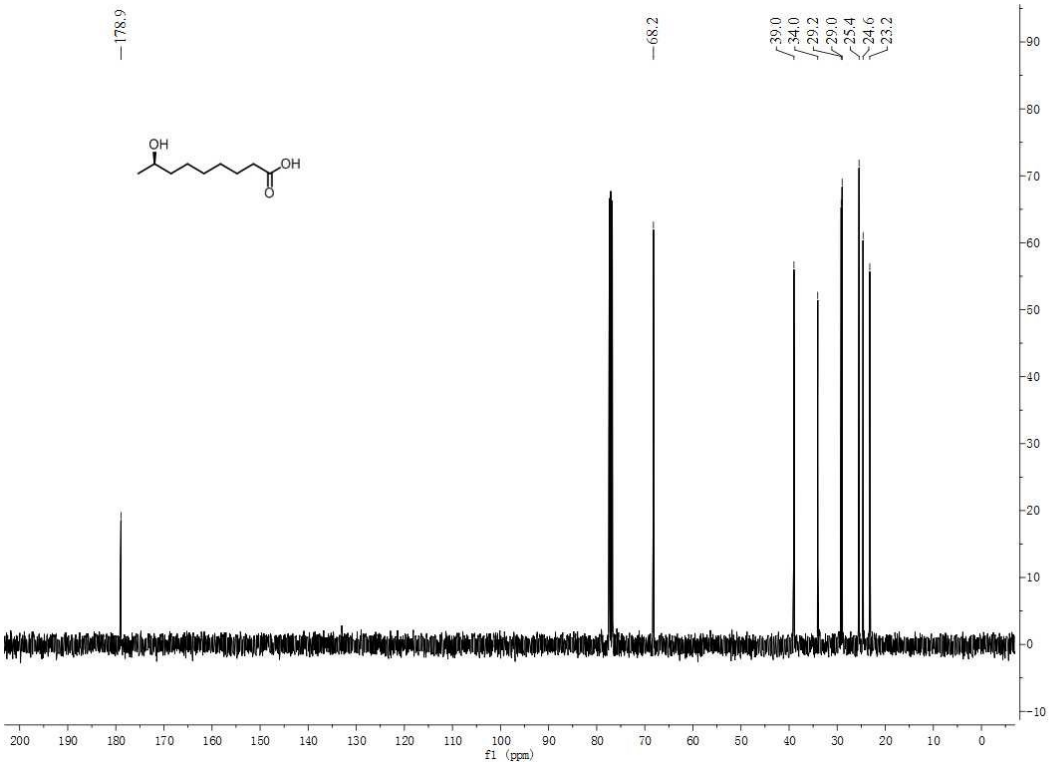


Figure S32. The ^{1}H NMR spectra copy of compound 11b in $CDCl_3$ (400 MHz)

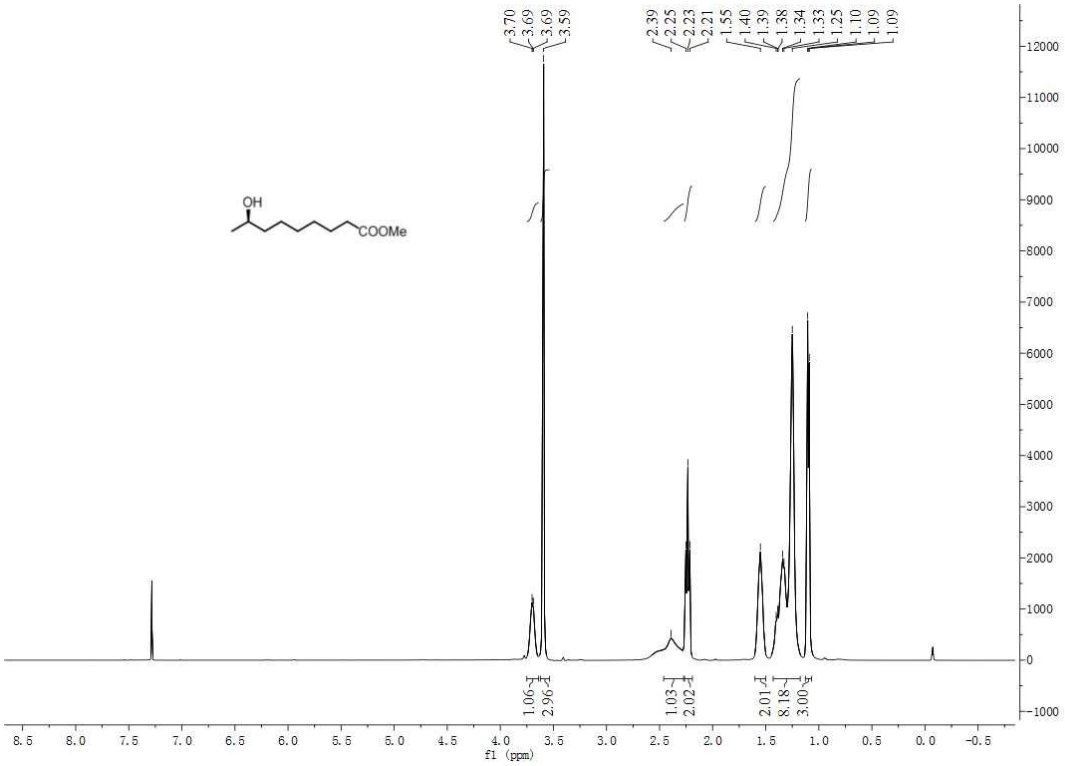


Figure S33. The ^{13}C NMR spectra copy of compound 11b in $CDCl_3$ (101 MHz)

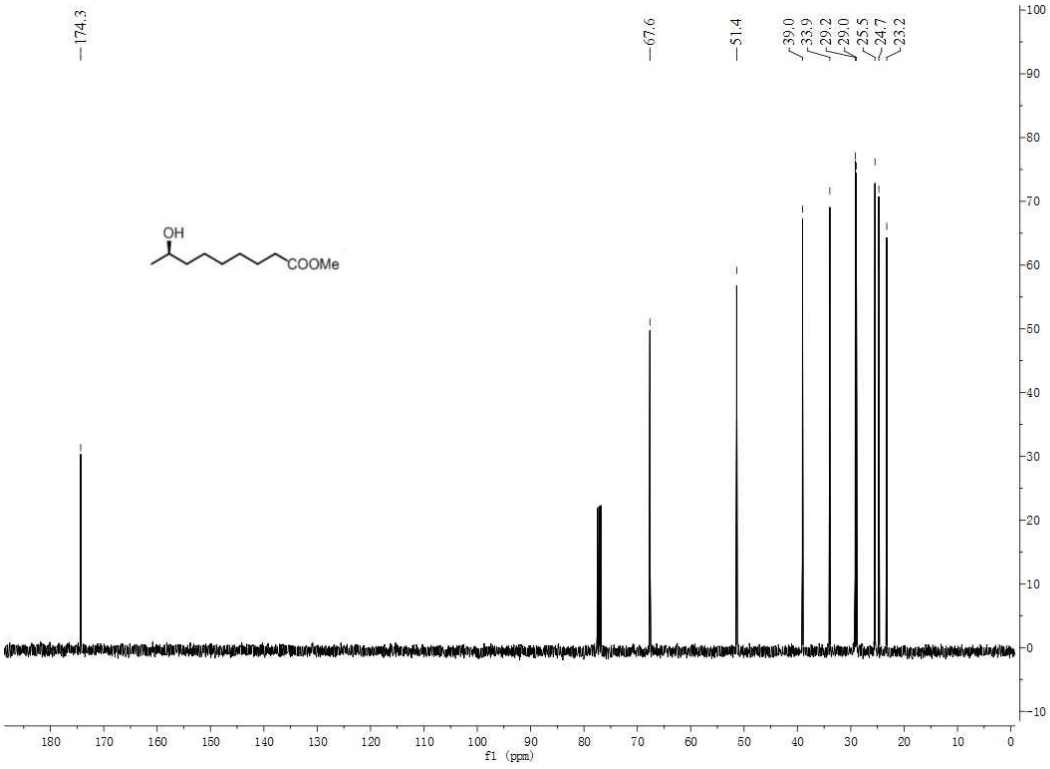


Figure S34. The ^{1}H NMR spectra copy of compound 11a in $CDCl_3$ (400 MHz)

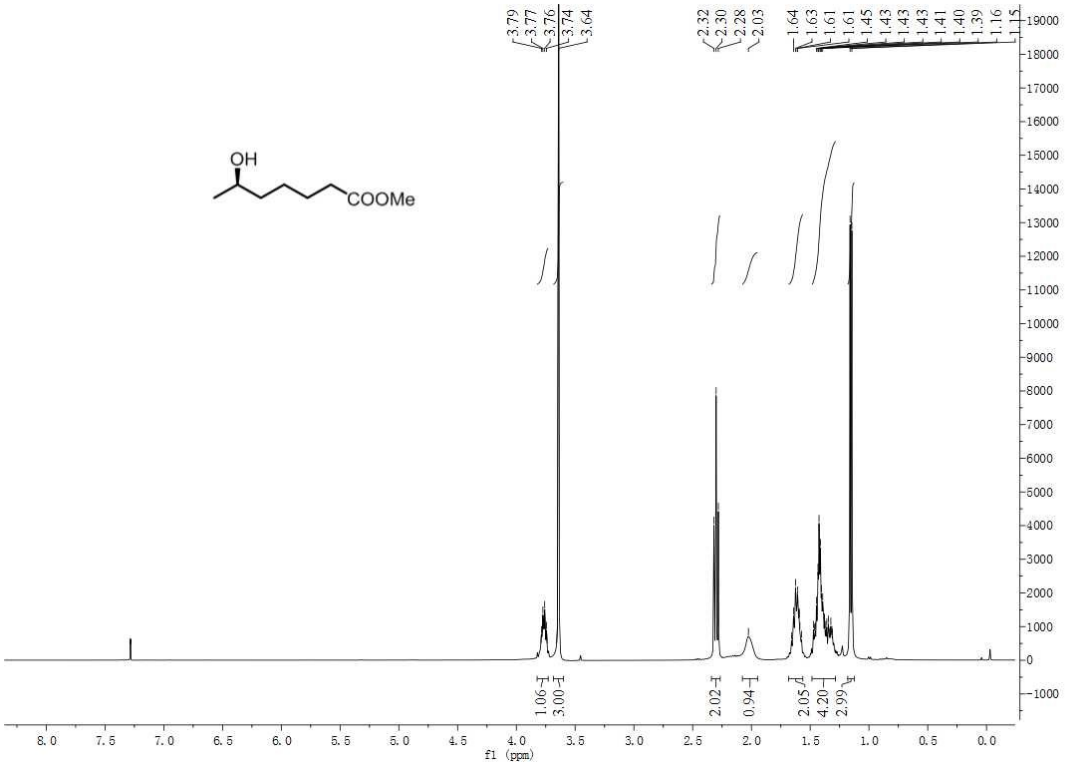


Figure S35. The ^{13}C NMR spectra copy of compound 11a in $CDCl_3$ (101 MHz)

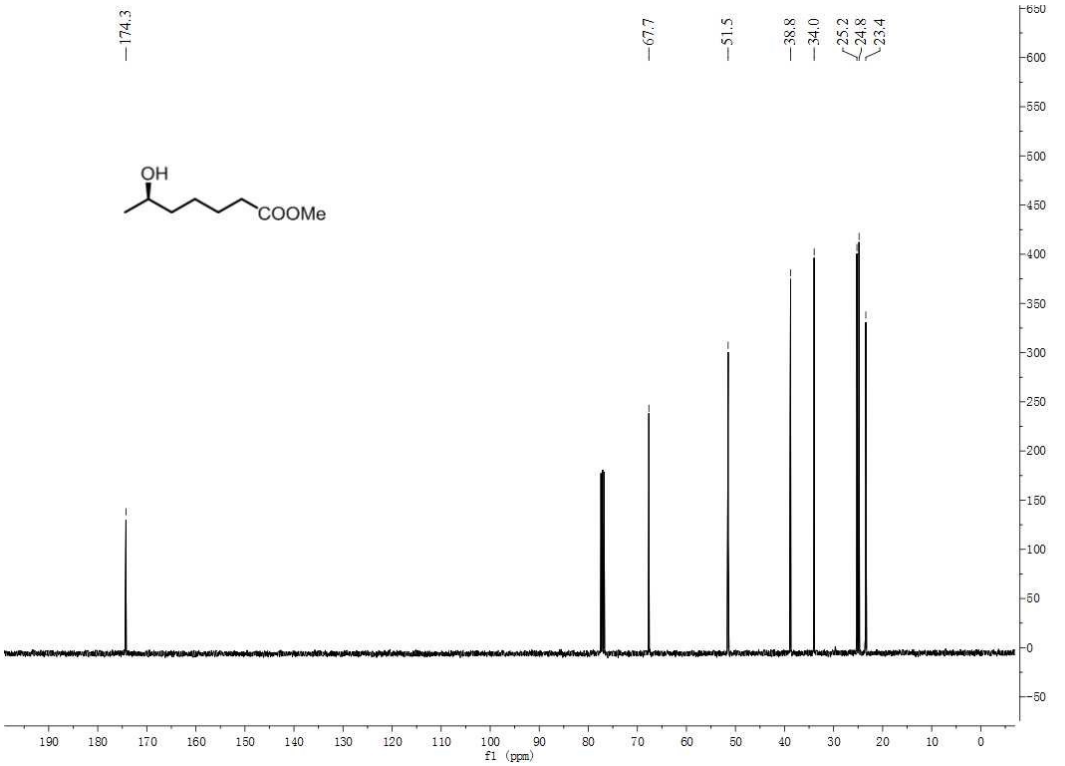


Figure S36. The ^{1}H NMR spectra copy of compound 11c in $CDCl_3$ (500 MHz)

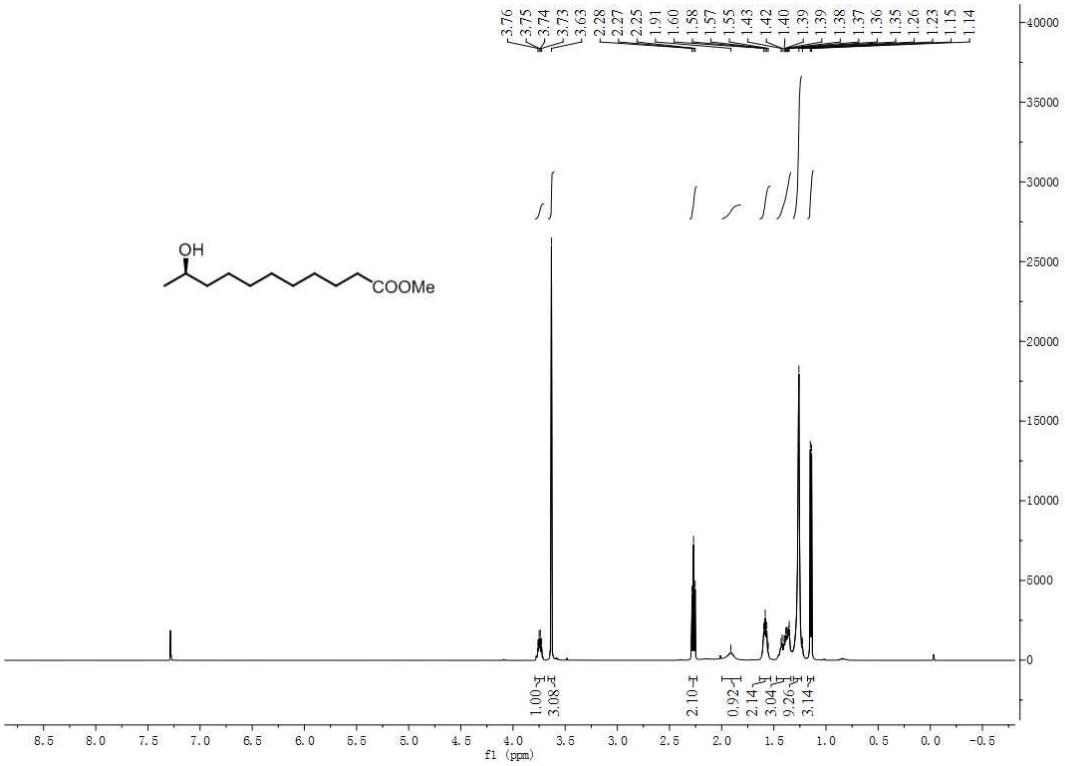


Figure S37. The ^{13}C-NMR spectra copy of compound 11c in $CDCl_3$ (126 MHz)

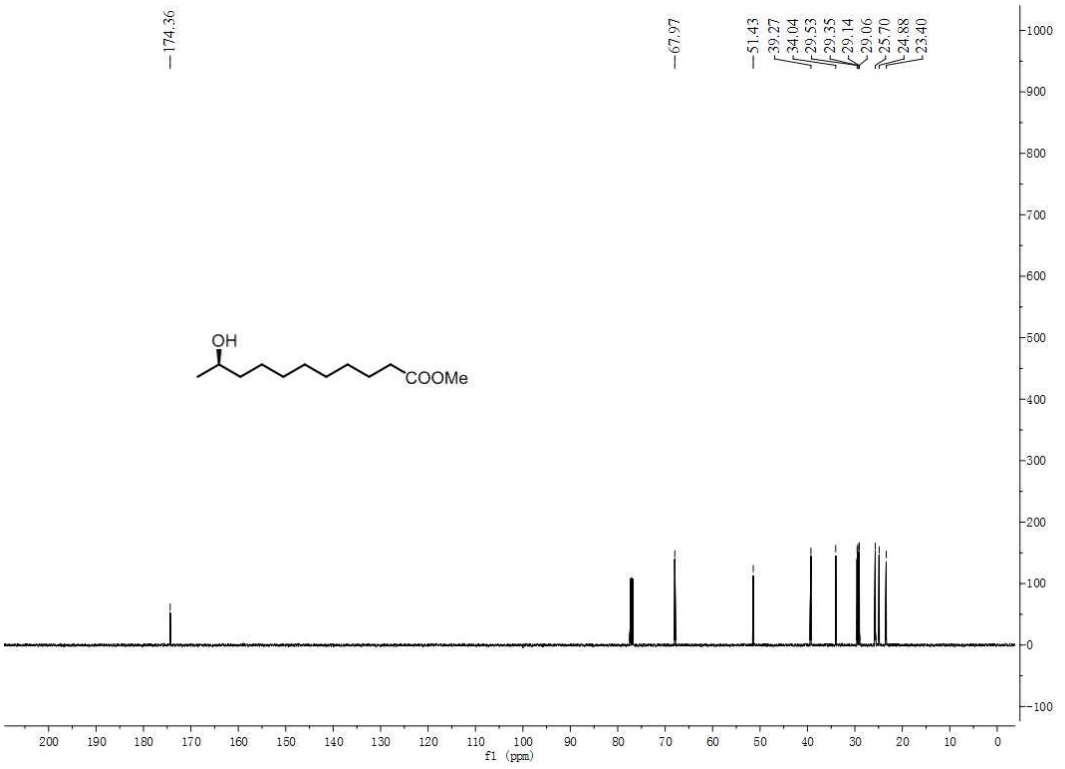


Figure S38. The ^{1}H NMR spectra copy of compound 11e in $CDCl_3$ (500 MHz)

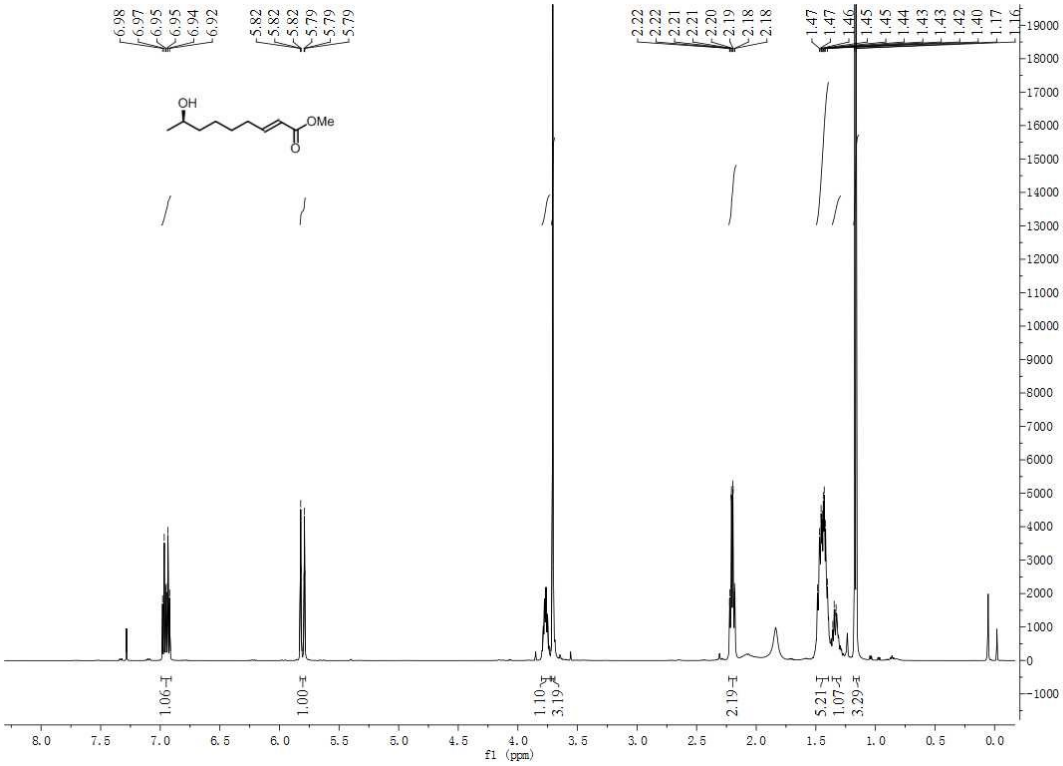


Figure S39. The ^{13}C NMR spectra copy of compound 11e in $CDCl_3$ (126 MHz)

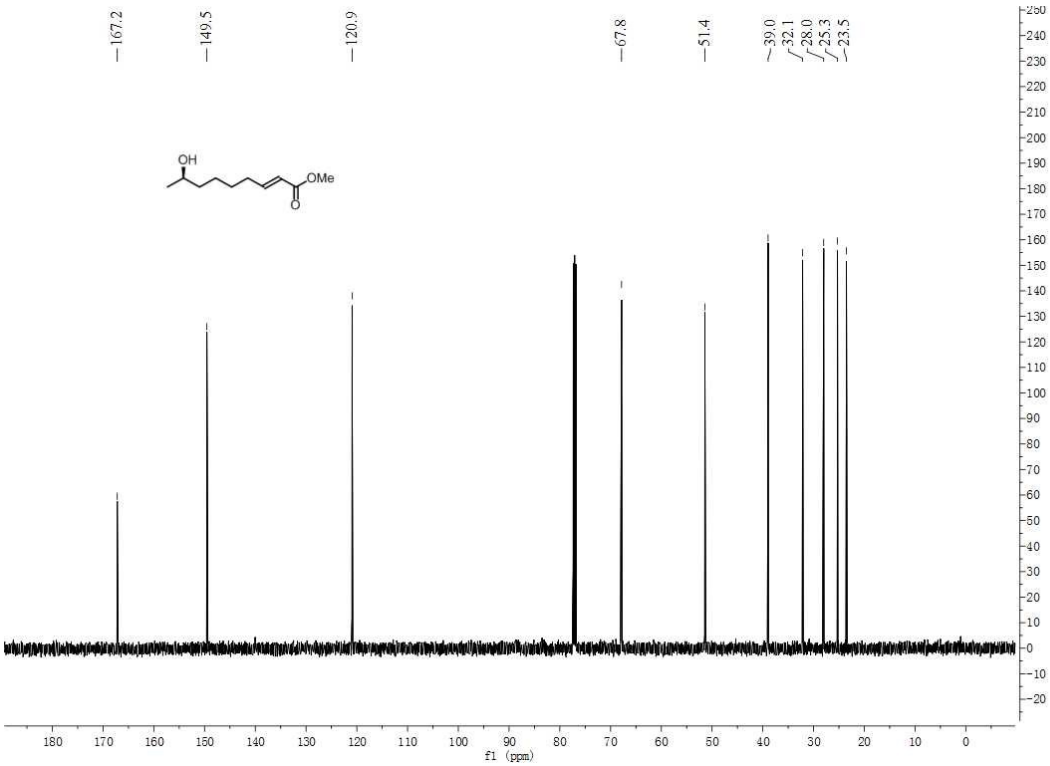


Figure S40. The ^{1}H NMR spectra copy of compound 12a in $CDCl_3$ (500 MHz)

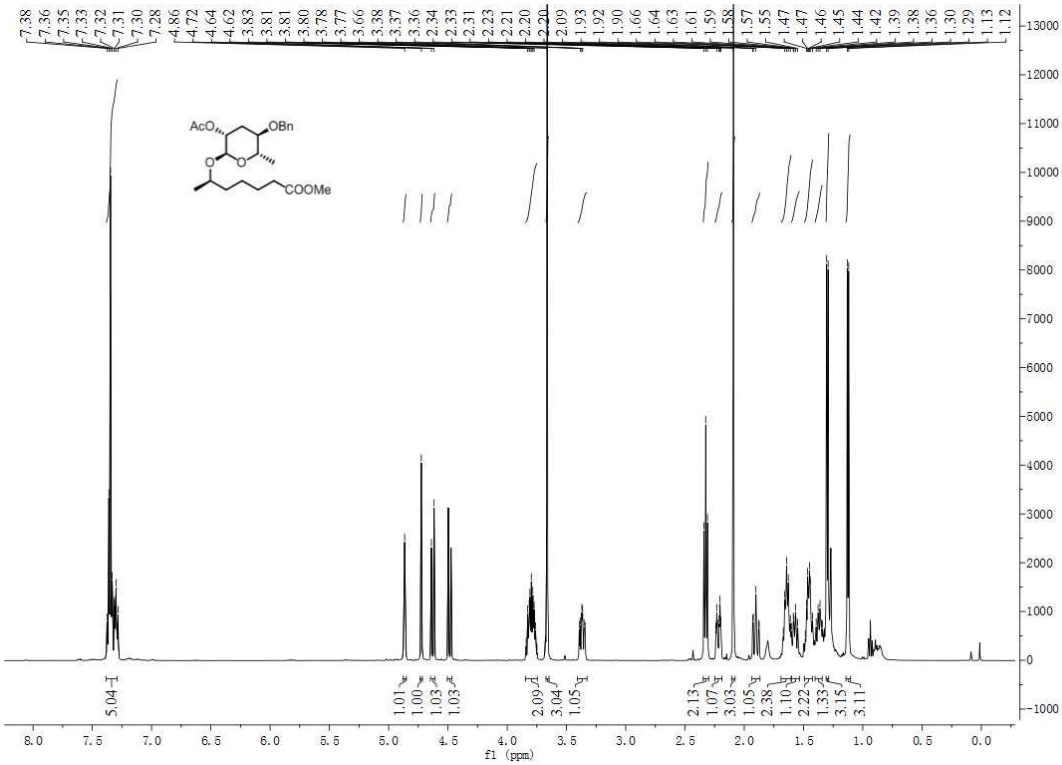


Figure S41. The ^{13}C NMR spectra copy of compound 12a in $CDCl_3$ (126 MHz)

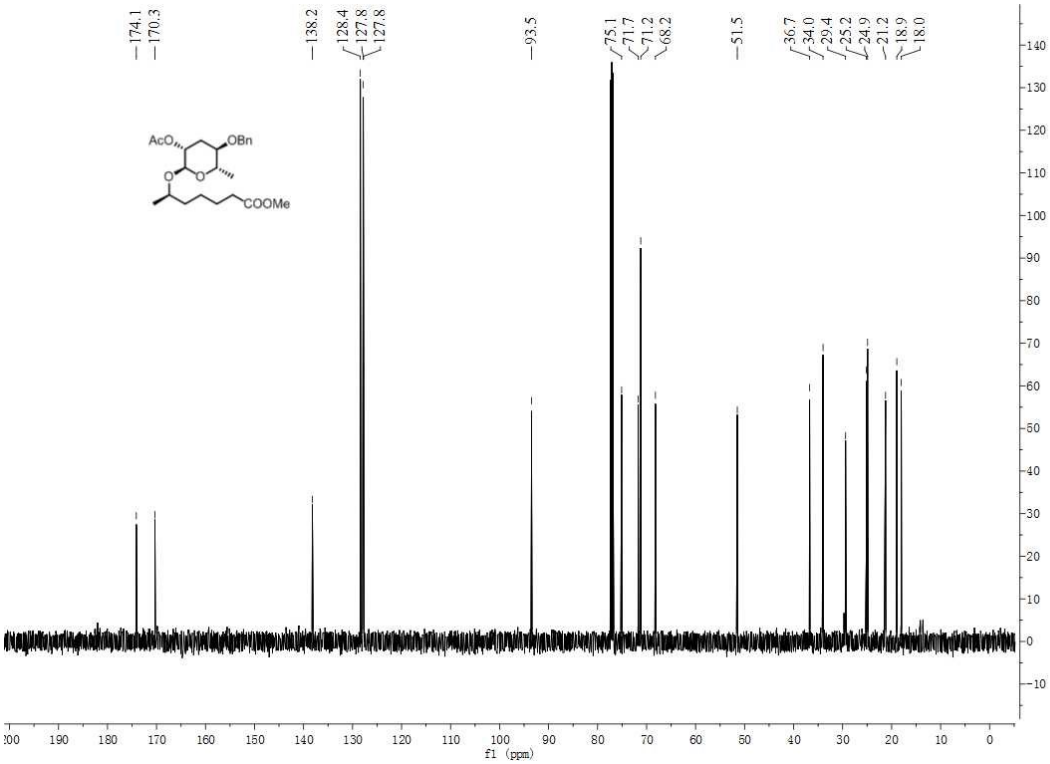


Figure S42. The ^{1}H NMR spectra copy of compound 13a in $CDCl_3$ (500 MHz)

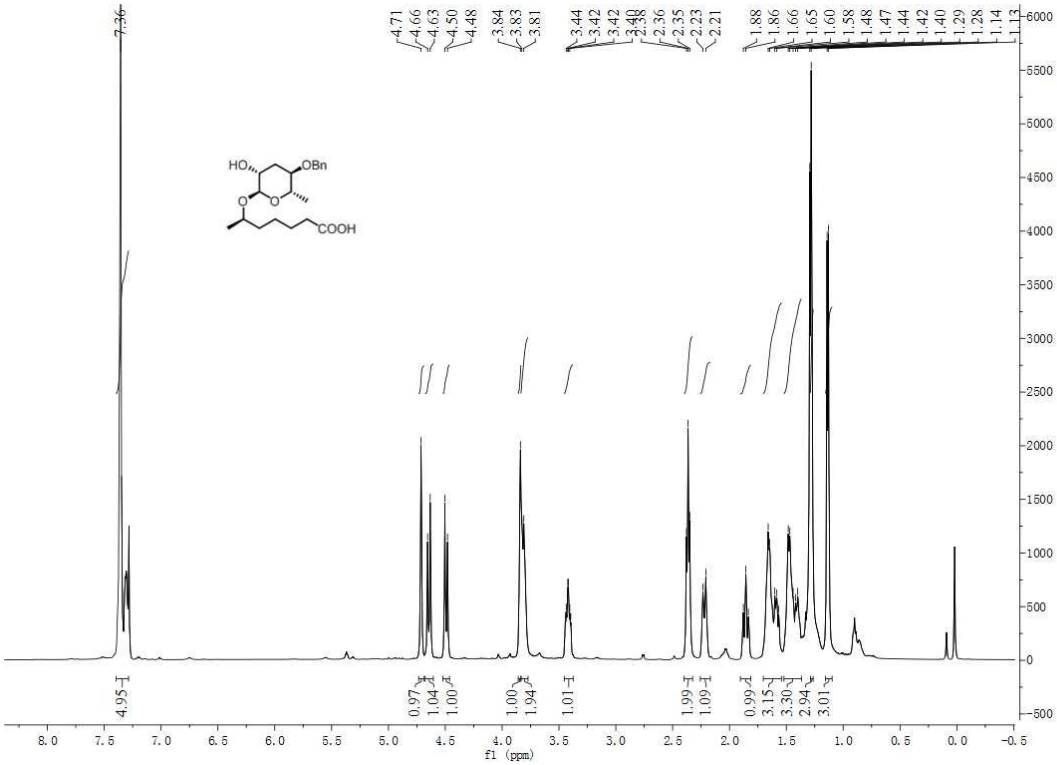


Figure S43. The ^{13}C NMR spectra copy of compound 13a in $CDCl_3$ (126 MHz)

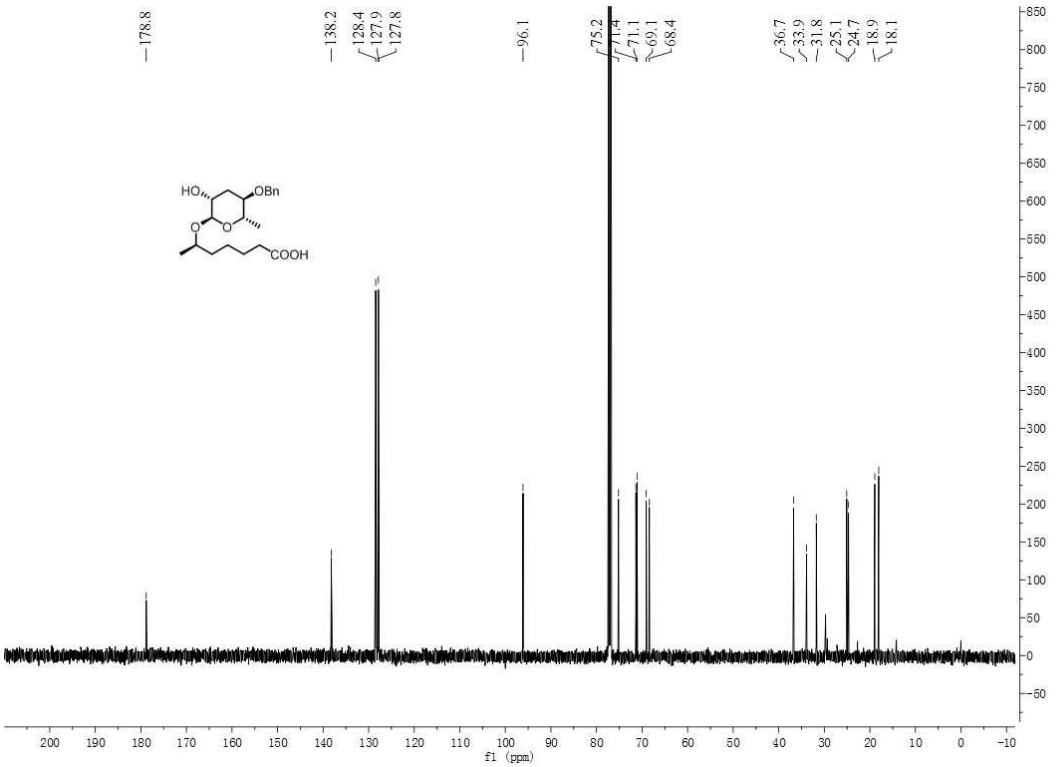


Figure S44. The ^{1}H NMR spectra copy of compound 14a (7) in $CDCl_3$ (400 MHz)

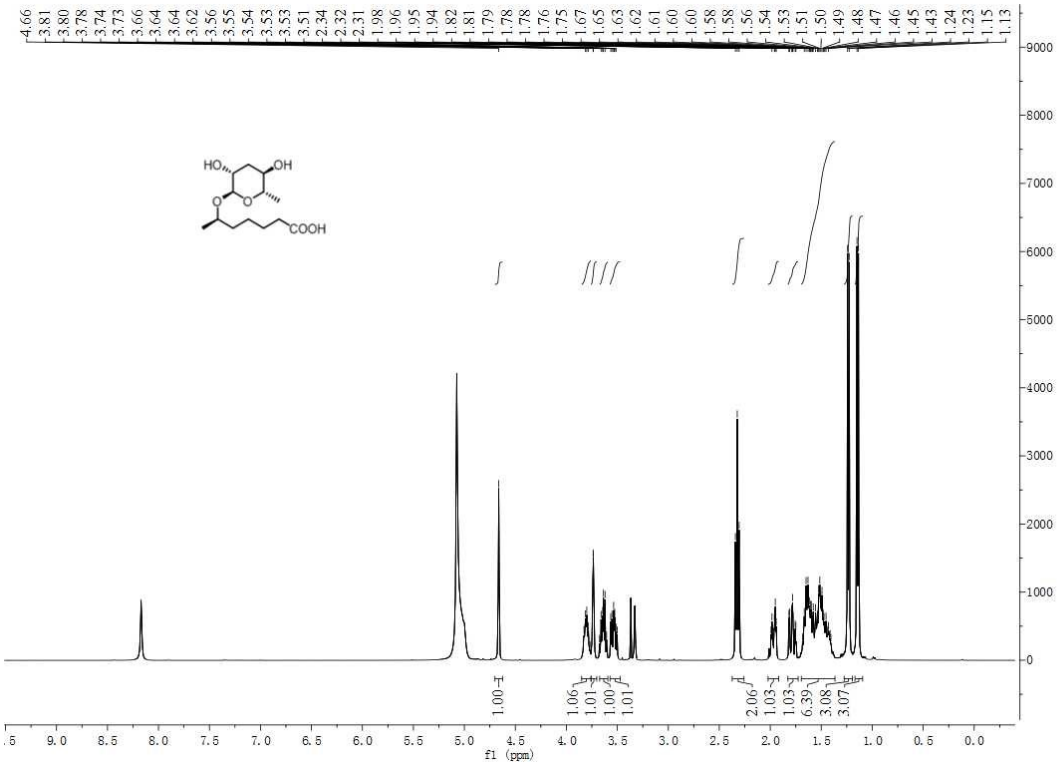


Figure S45. The ^{13}C NMR spectra copy of compound 14a (7) in $CDCl_3$ (101 MHz)

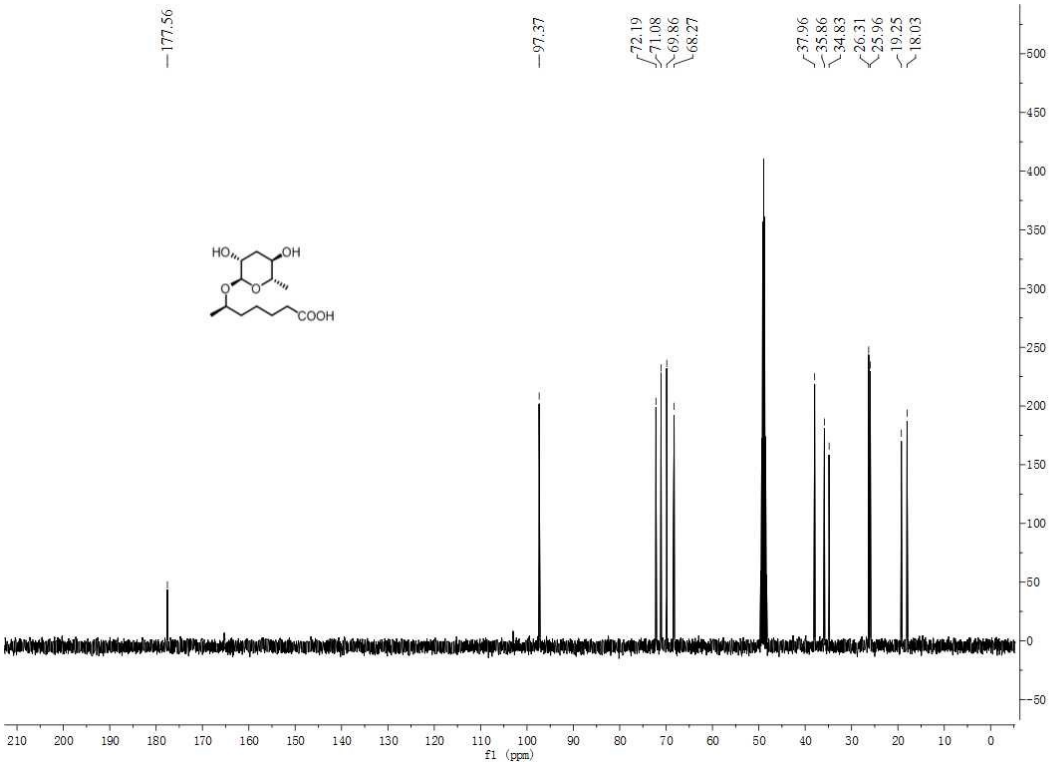


Figure S46. The ^{1}H NMR spectra copy of compound 12b in $CDCl_3$ (500 MHz)

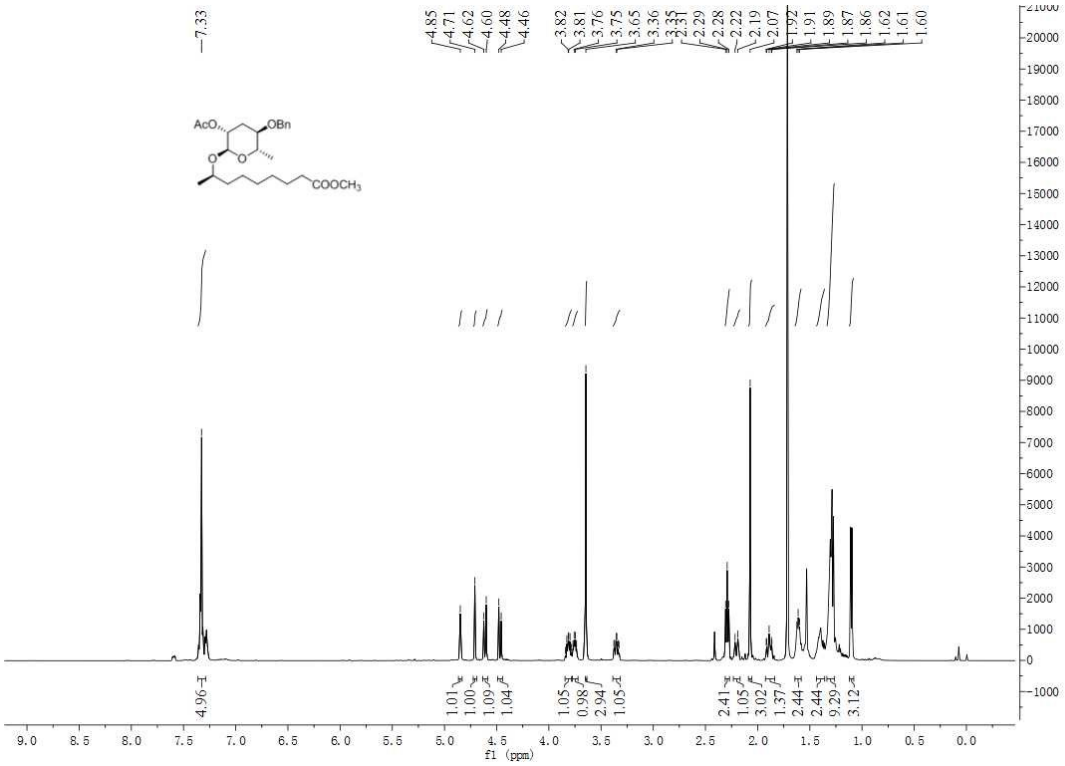


Figure S47. The ^{13}C NMR spectra copy of compound 12b in $CDCl_3$ (126 MHz)

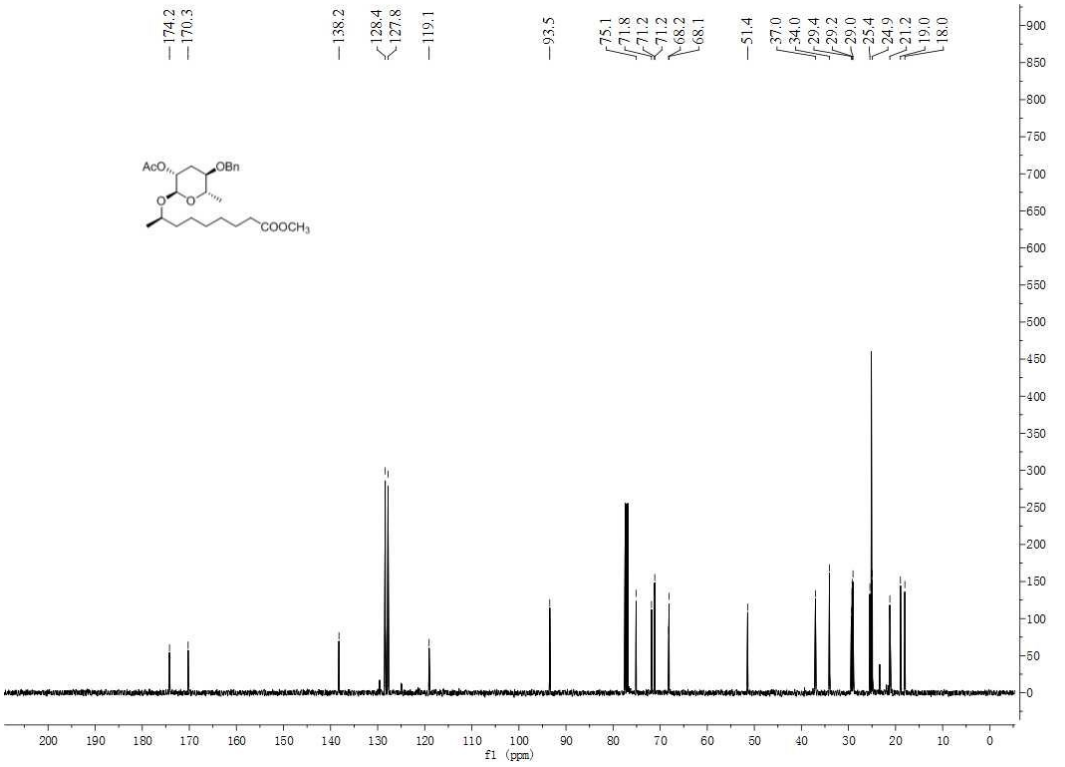


Figure S48. The ^{1}H NMR spectra copy of compound 13b in $CDCl_3$ (500 MHz)

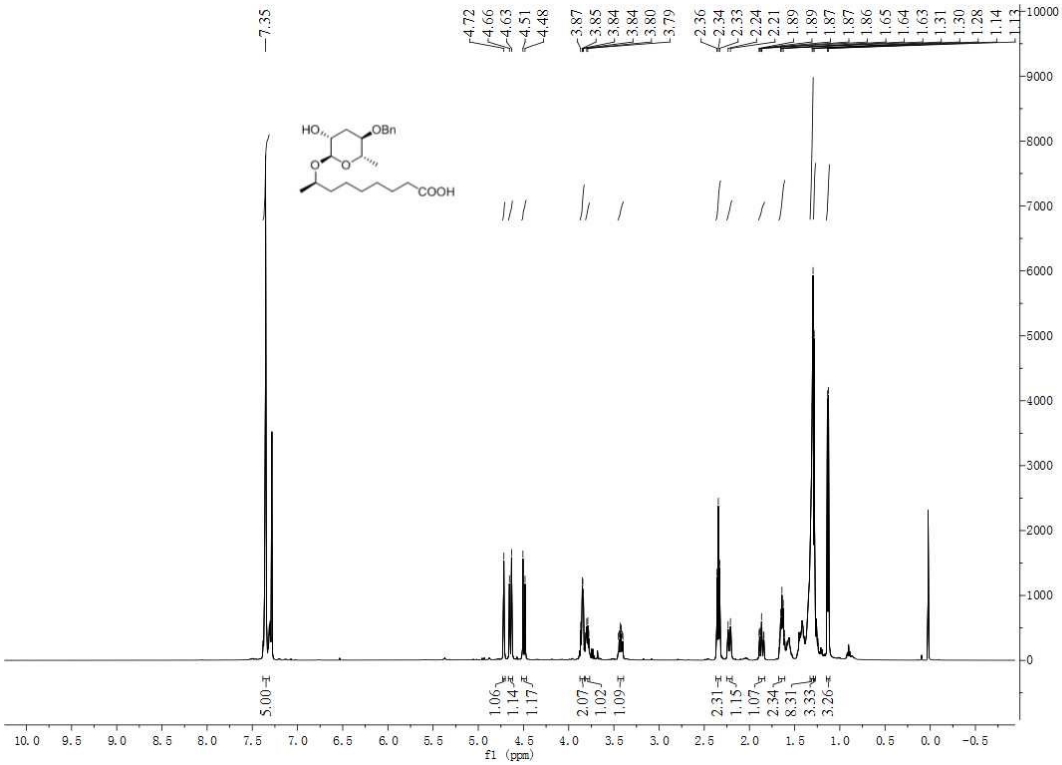


Figure S49. The ^{13}C NMR spectra copy of compound 13b in $CDCl_3$ (126 MHz)

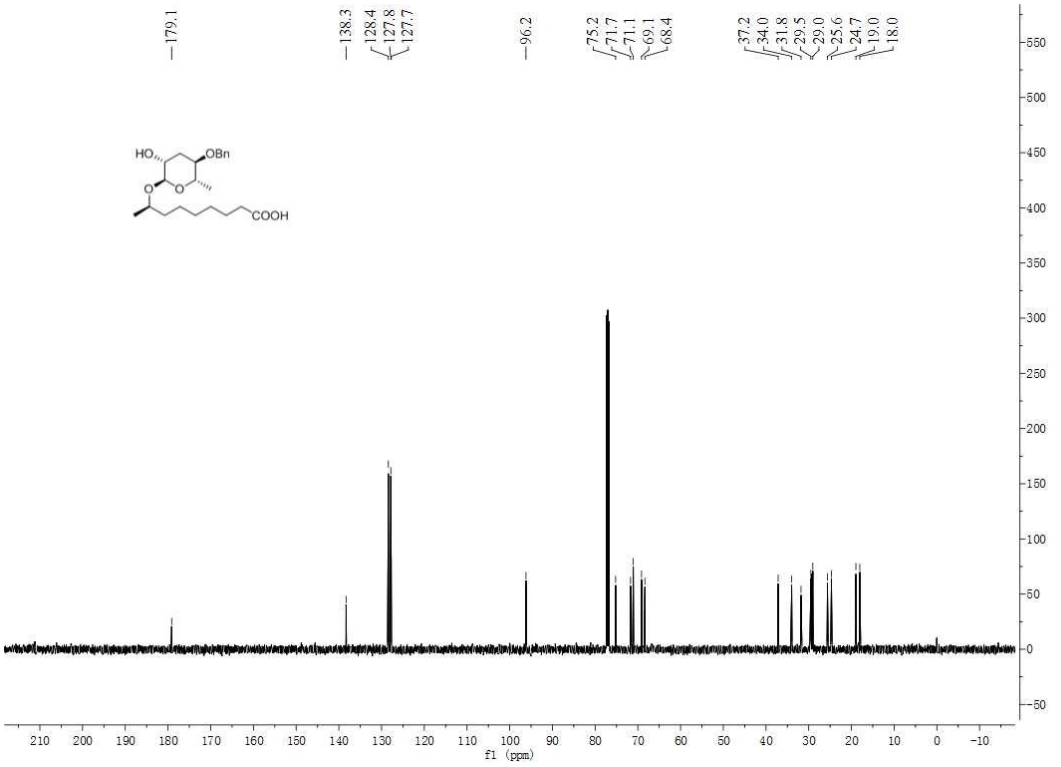


Figure S50. The ^{1}H NMR spectra copy of compound 14b in CD_3OD (400 MHz)

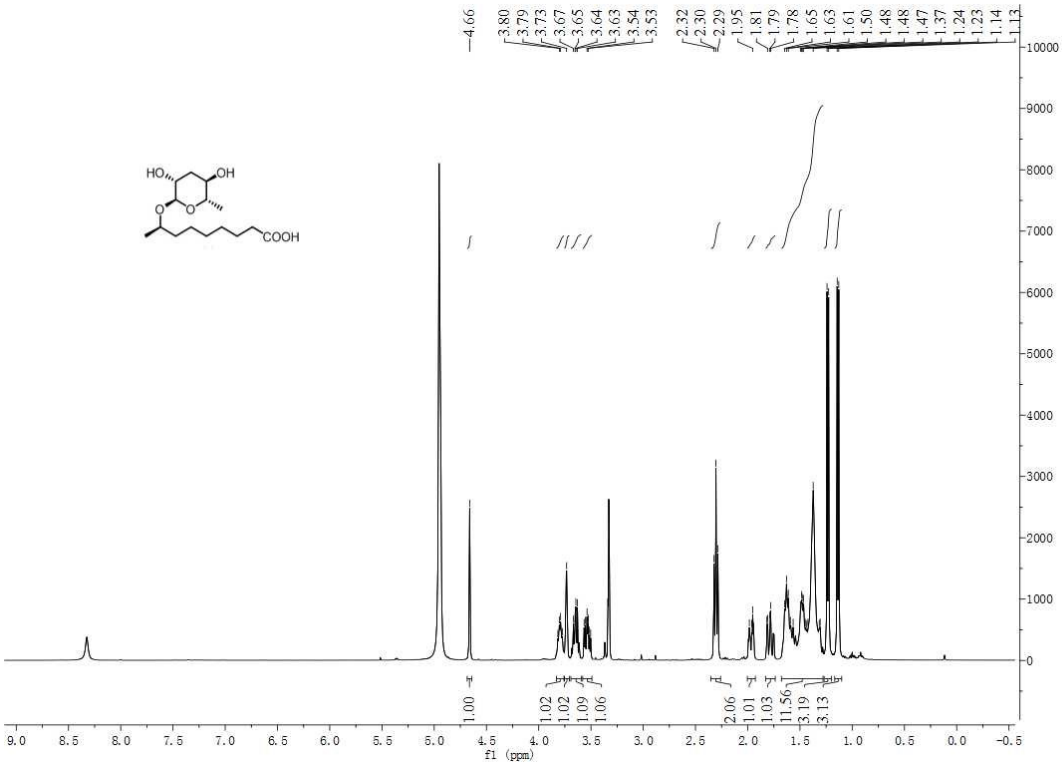


Figure S51. The ^{13}C NMR spectra copy of compound 14b in CD_3OD (101 MHz)

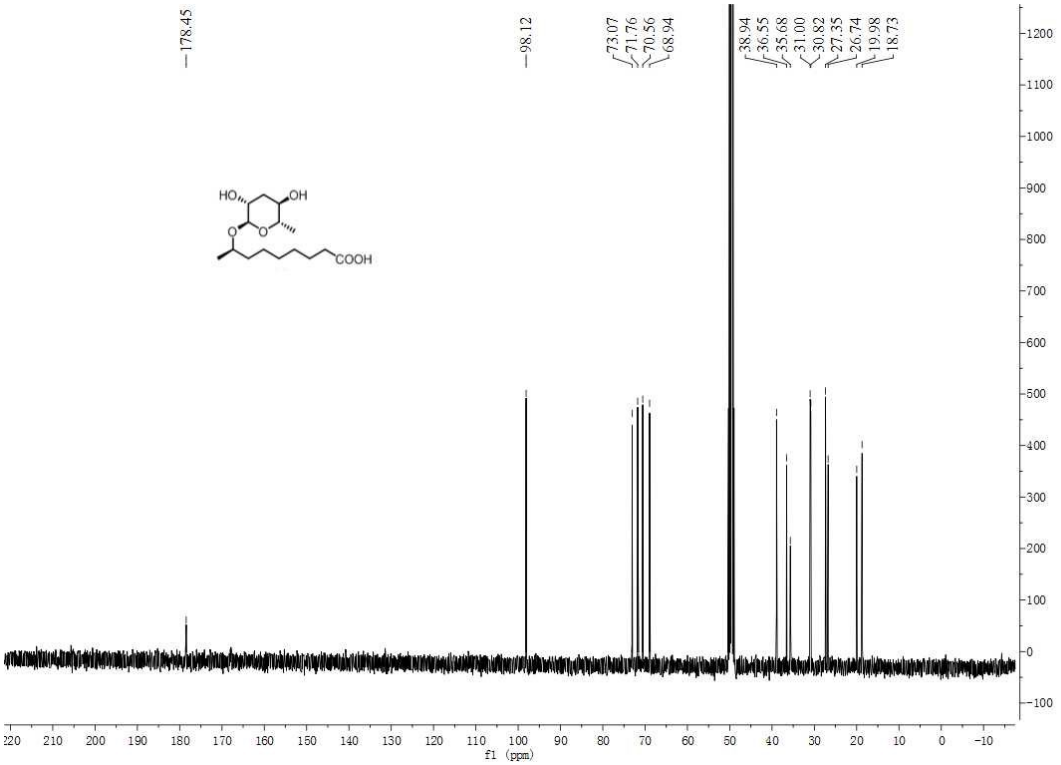


Figure S52. The ^{1}H NMR spectra copy of compound 12c in $CDCl_3$ (400 MHz)

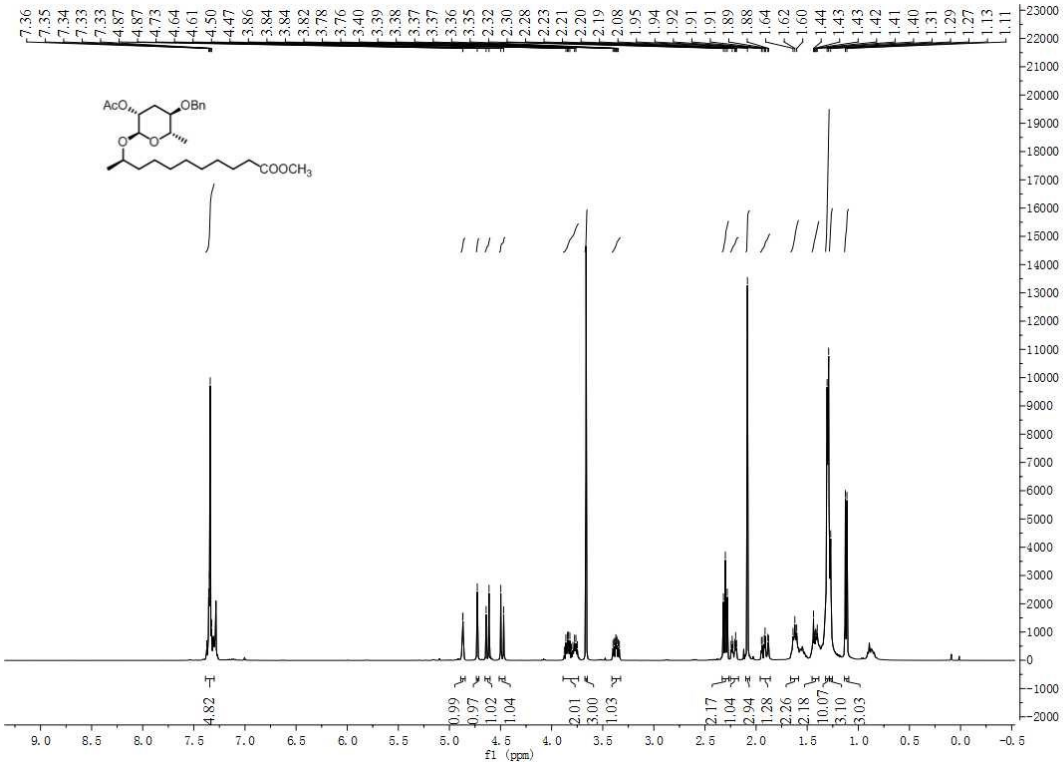


Figure S53. The ^{13}C NMR spectra copy of compound 12c in $CDCl_3$ (101 MHz)

Figure S54. The ^{1}H NMR spectra copy of compound 13c in $CDCl_3$ (500 MHz)

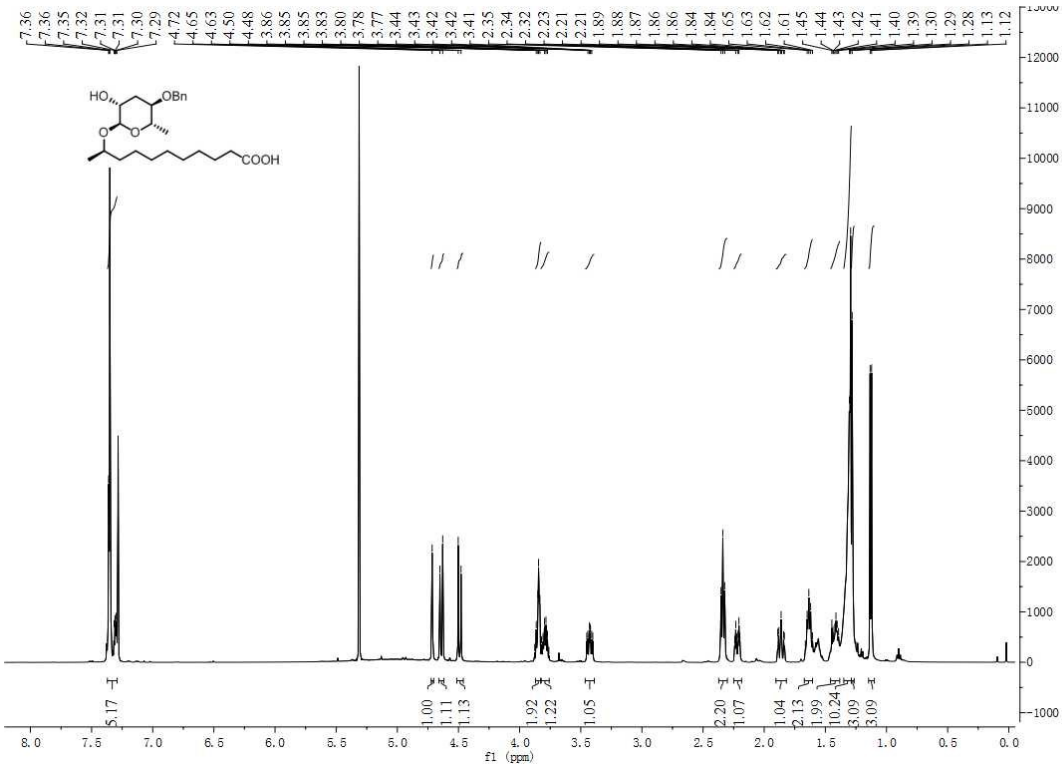


Figure S55. The ^{13}C NMR spectra copy of compound 13c in $CDCl_3$ (126 MHz)

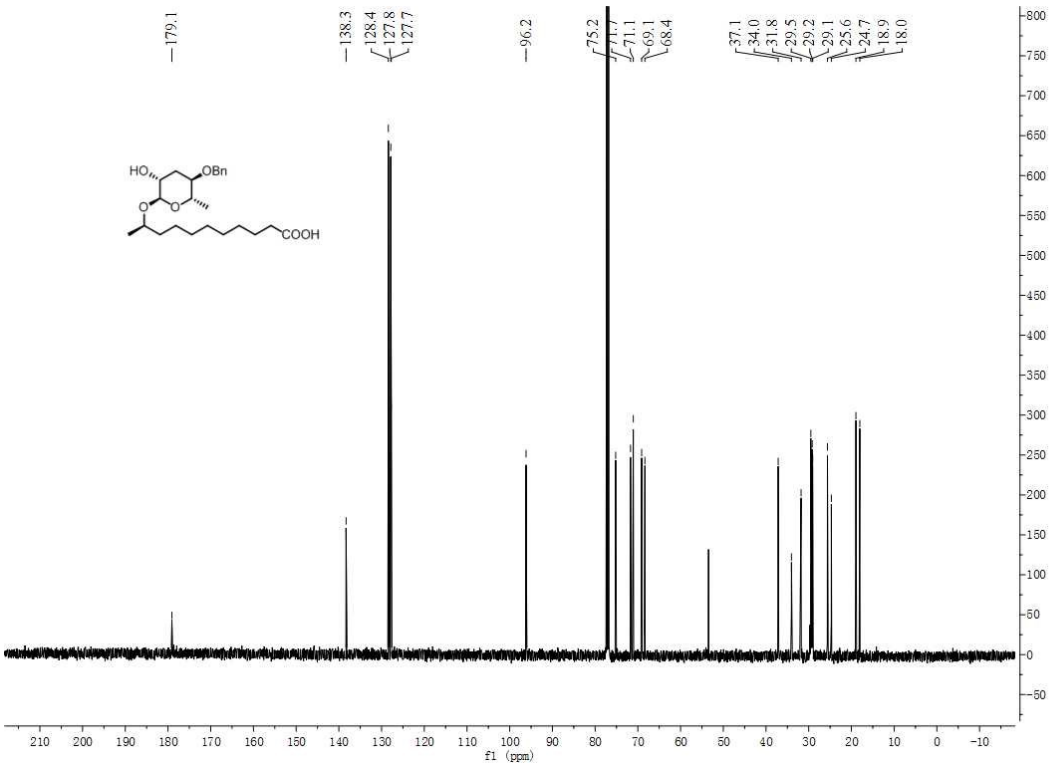


Figure S56. The ^{1}H NMR spectra copy of compound 14c in CD_3OD (500 MHz)

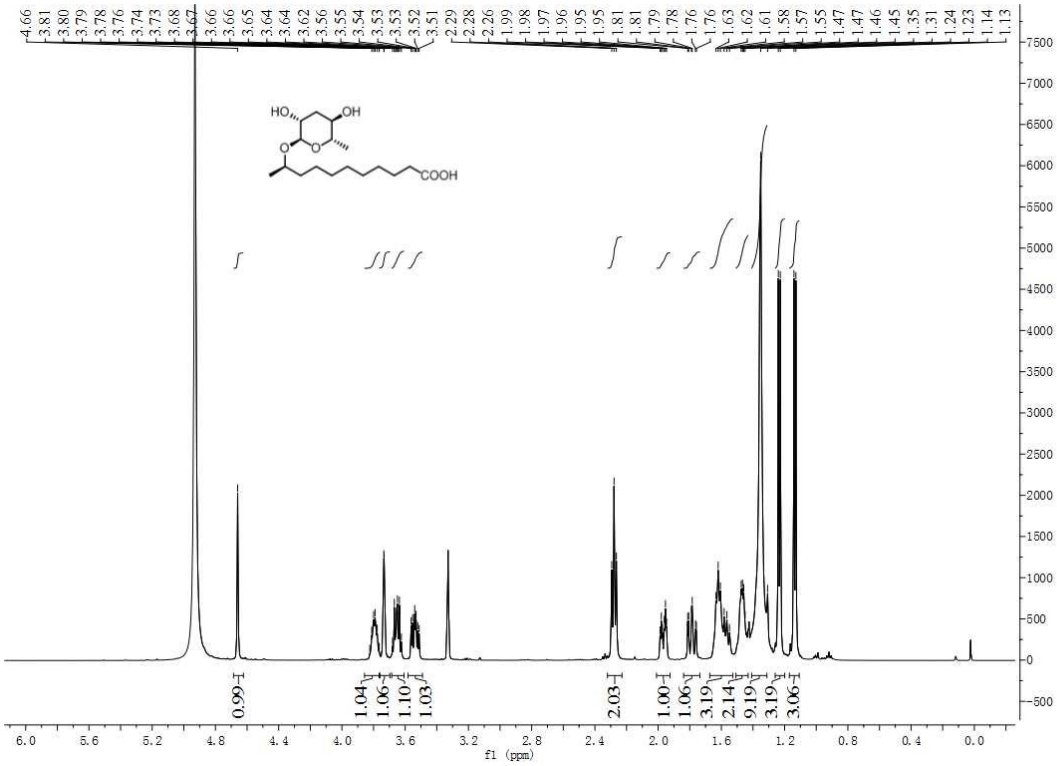


Figure S57. The ^{13}C NMR spectra copy of compound 14c in CD_3OD (126 MHz)

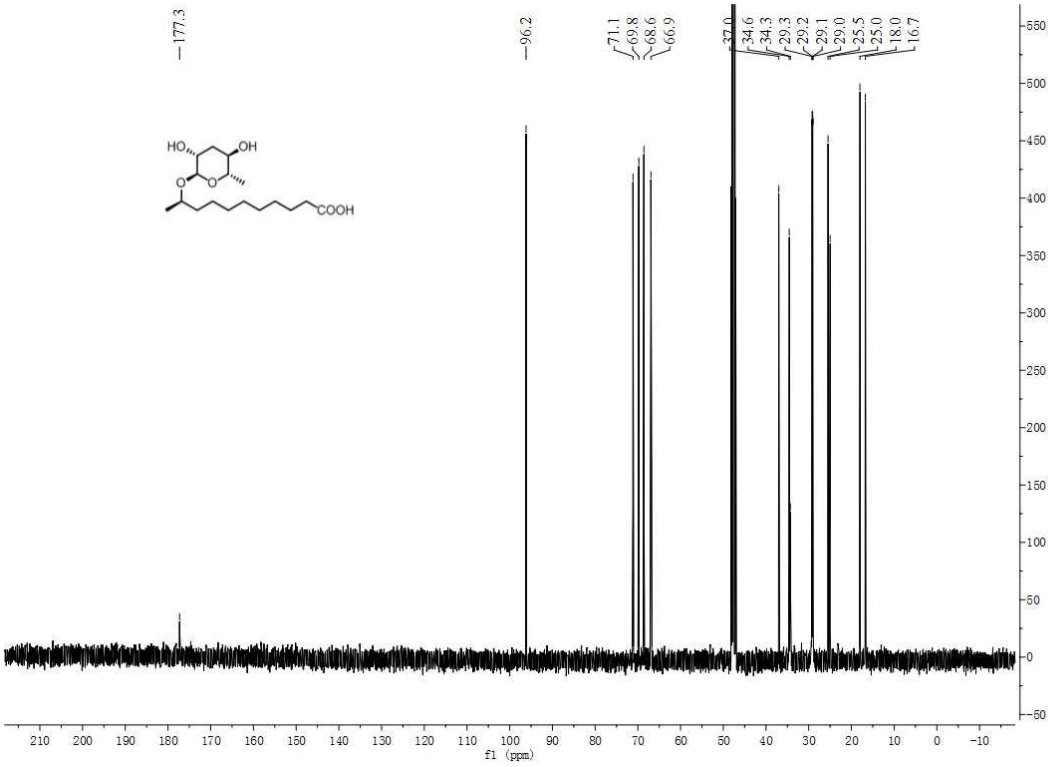


Figure S58. The ^{1}H NMR spectra copy of compound 12d in $CDCl_3$ (500 MHz)

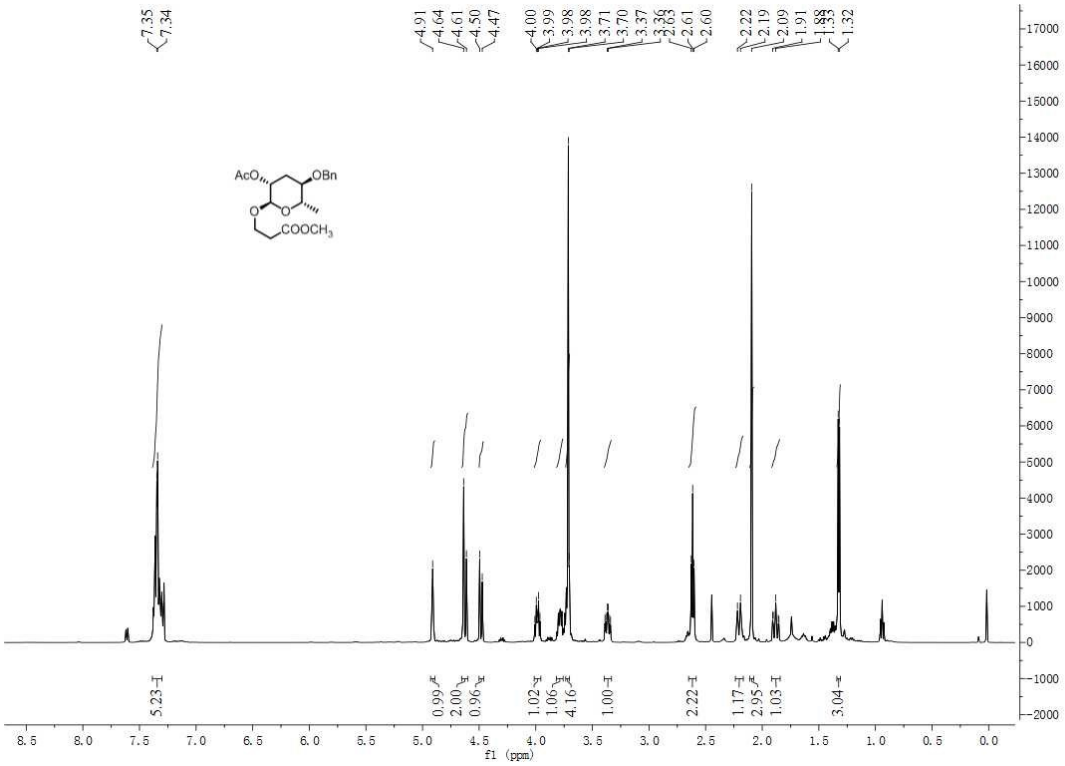


Figure S59. The ^{13}C NMR spectra copy of compound 12d in $CDCl_3$ (126 MHz)

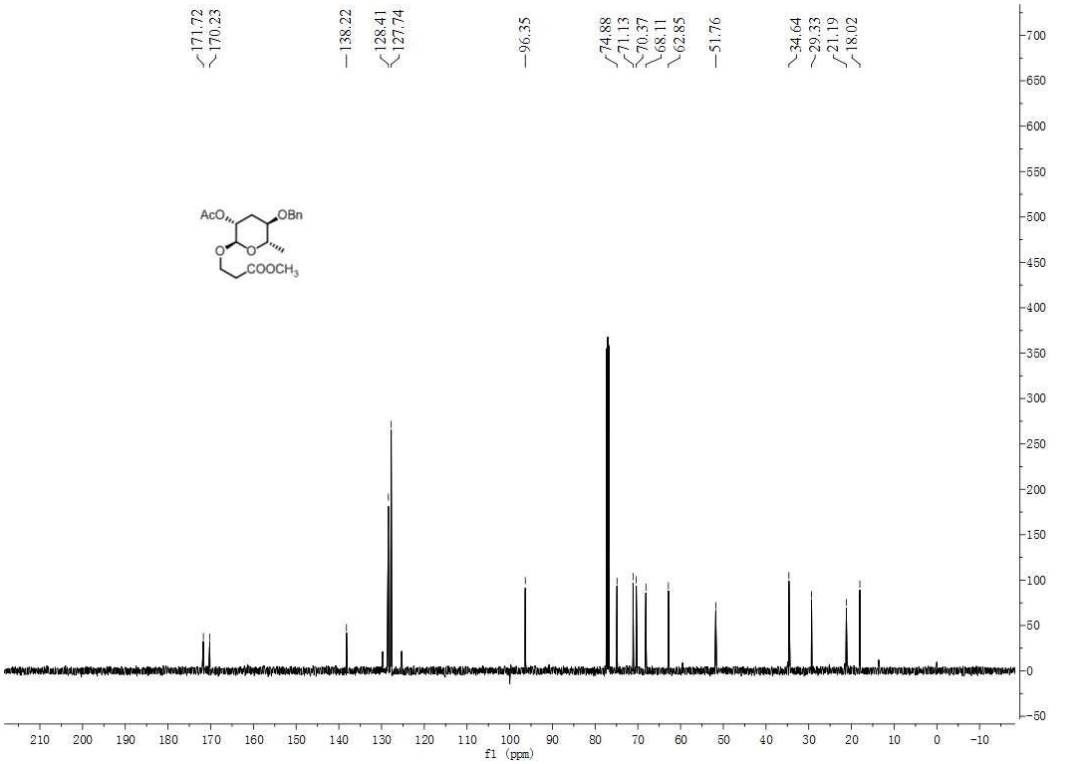


Figure S60. The ^{1}H NMR spectra copy of compound 13d in $CDCl_3$ (500 MHz)

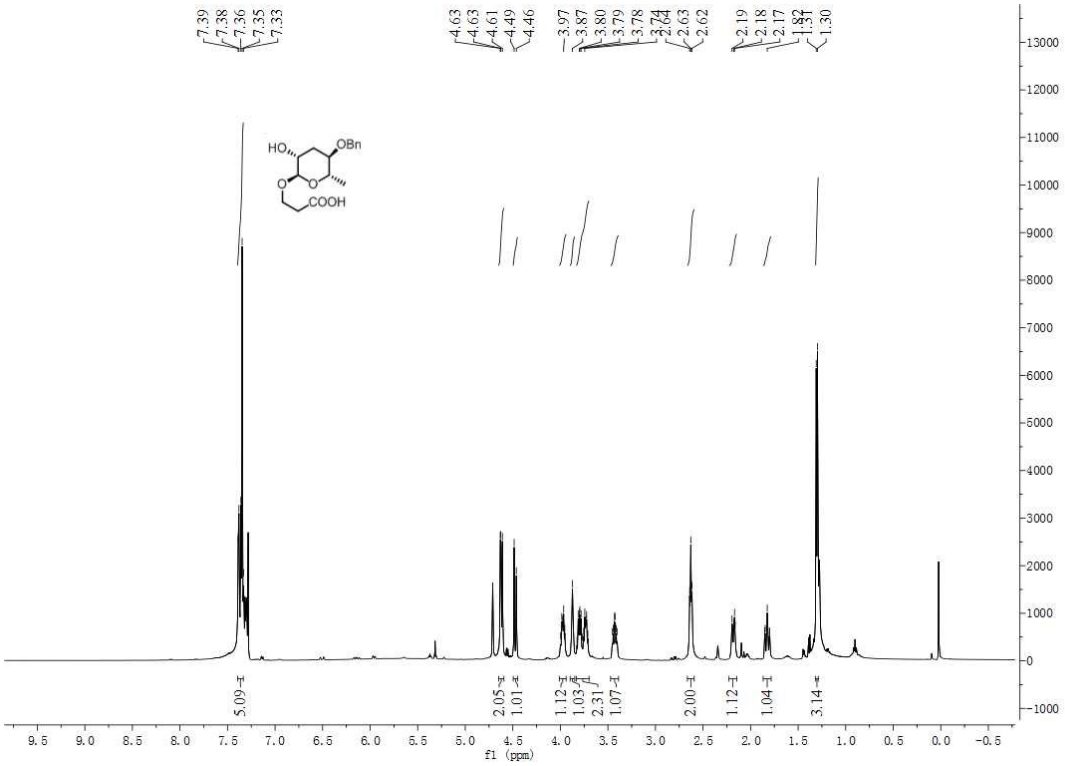


Figure S61. The ^{13}C NMR spectra copy of compound 13d in $CDCl_3$ (126 MHz)

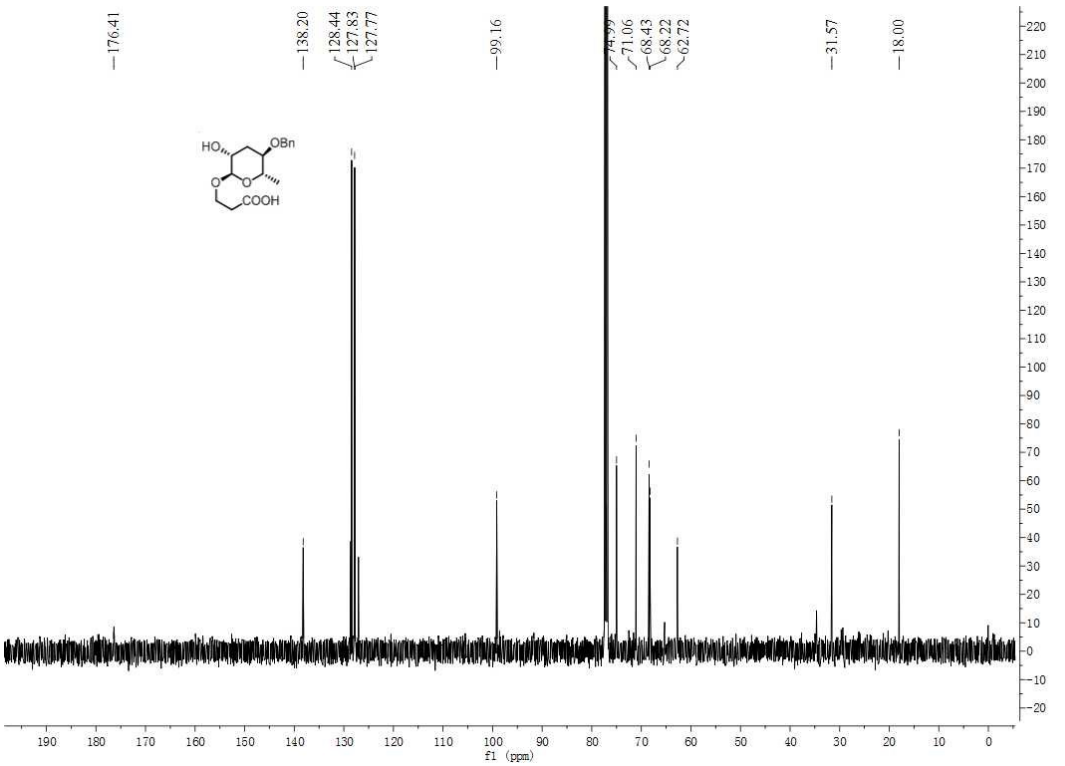


Figure S62. The ^{1}H NMR spectra copy of compound 14d in CD_3OD (500 MHz)

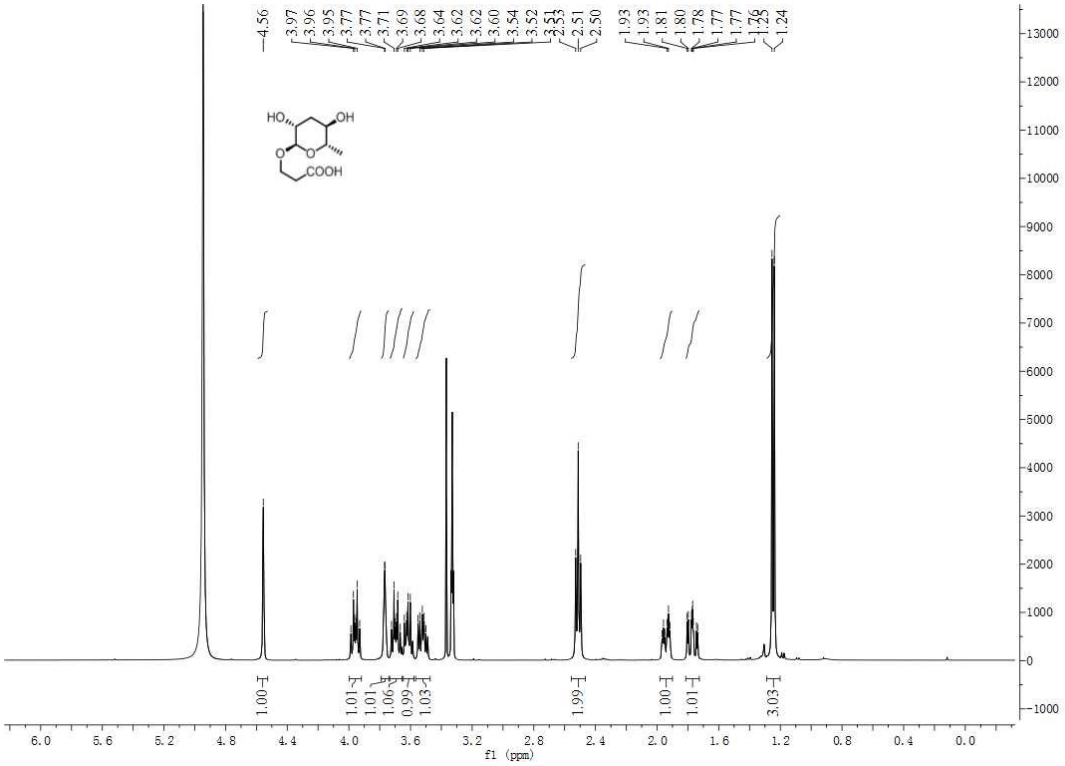


Figure S63. The ^{13}C NMR spectra copy of compound 14d in CD_3OD (126 MHz)

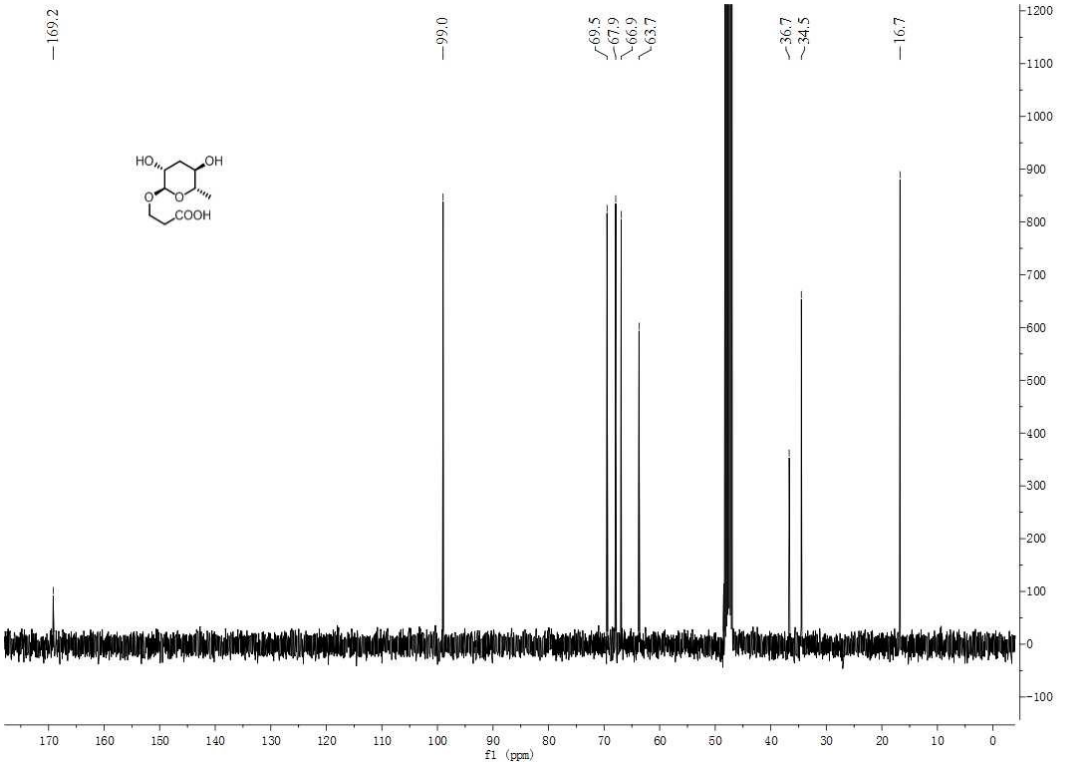


Figure S64. The ^{1}H NMR spectra copy of compound 15 in $CDCl_3$ (500 MHz)

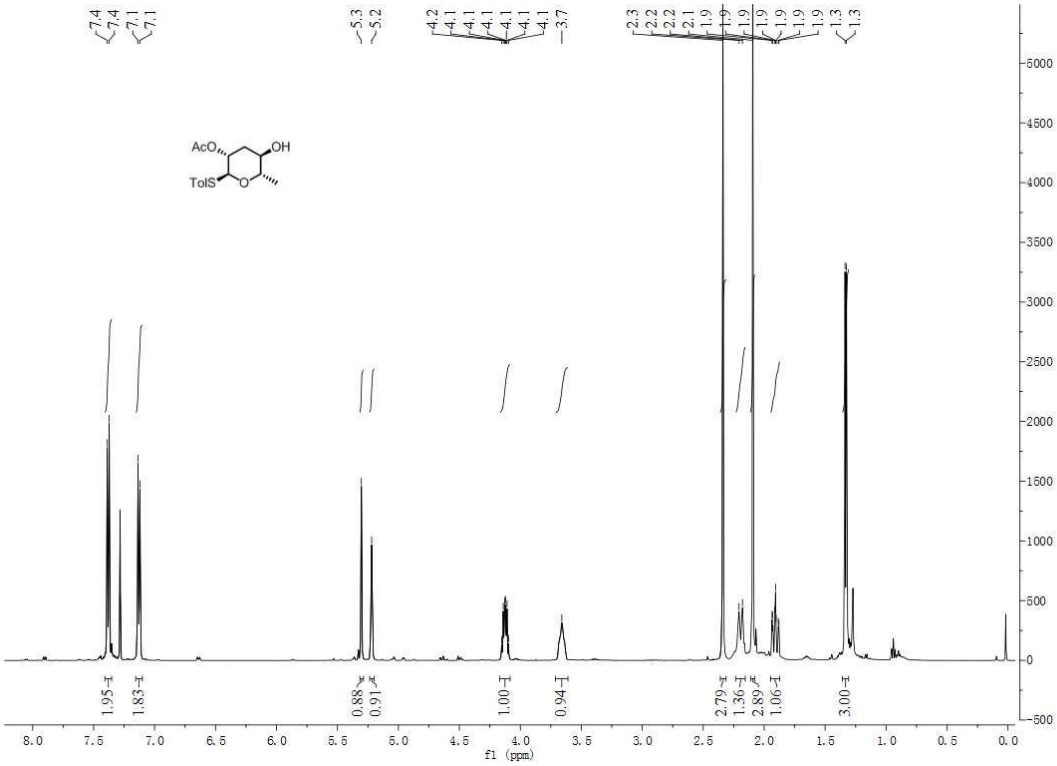


Figure S65. The ^{13}C NMR spectra copy of compound 15 in $CDCl_3$ (126 MHz)

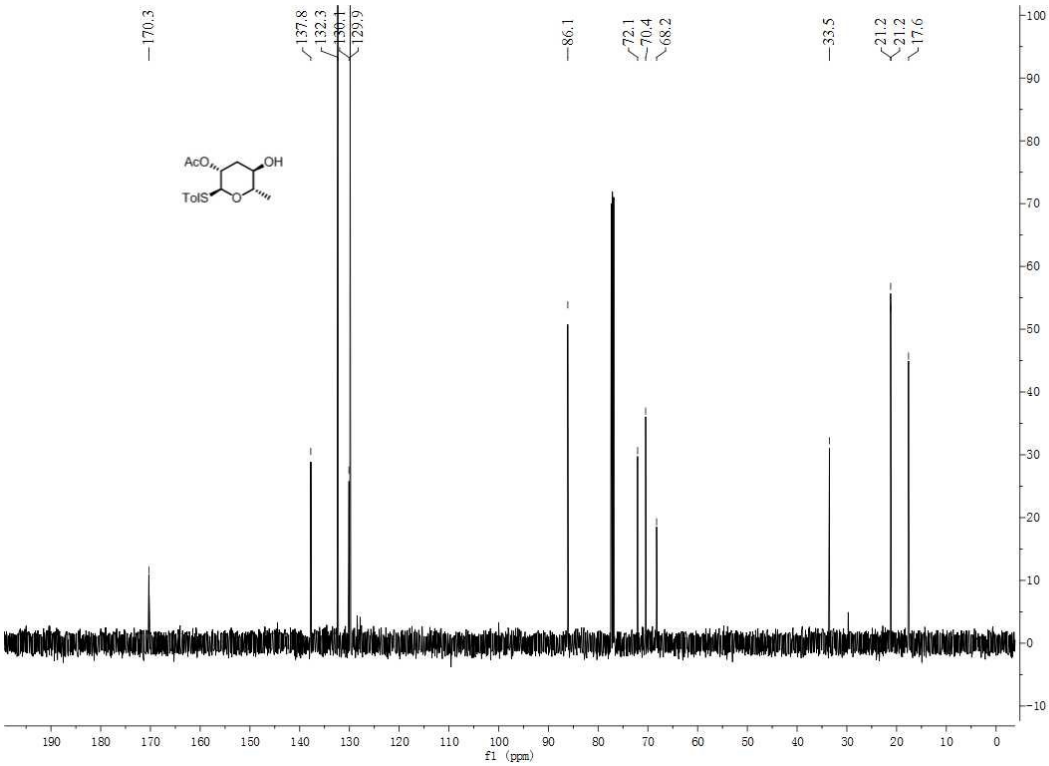


Figure S66. The ^{1}H NMR spectra copy of compound 16a in $CDCl_3$ (500 MHz)

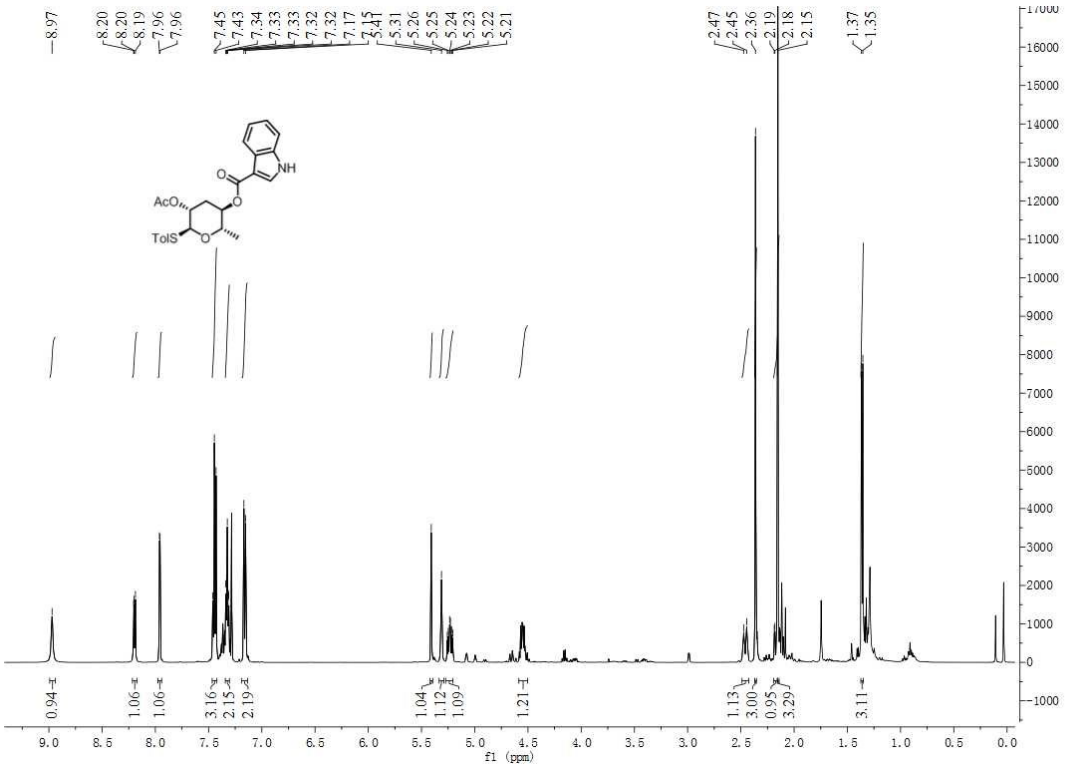


Figure S67. The ^{13}C NMR spectra copy of compound 16a in $CDCl_3$ (126 MHz)

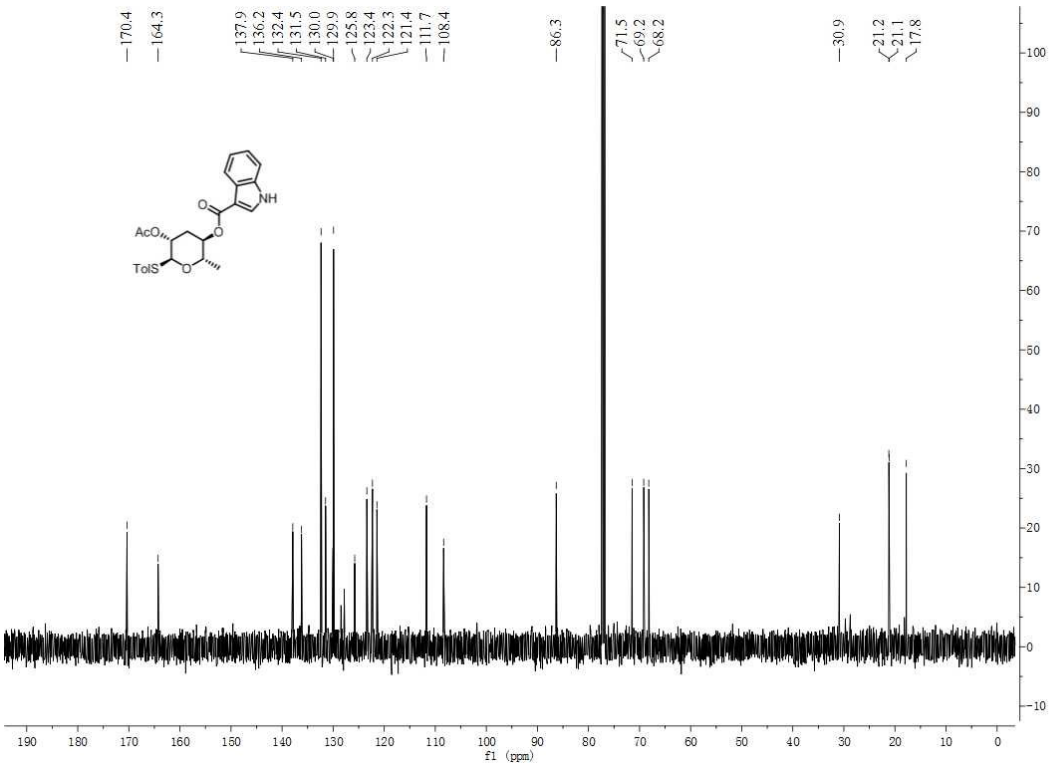


Figure S68. The ^{1}H NMR spectra copy of compound 17a in $CDCl_3$ (500 MHz)

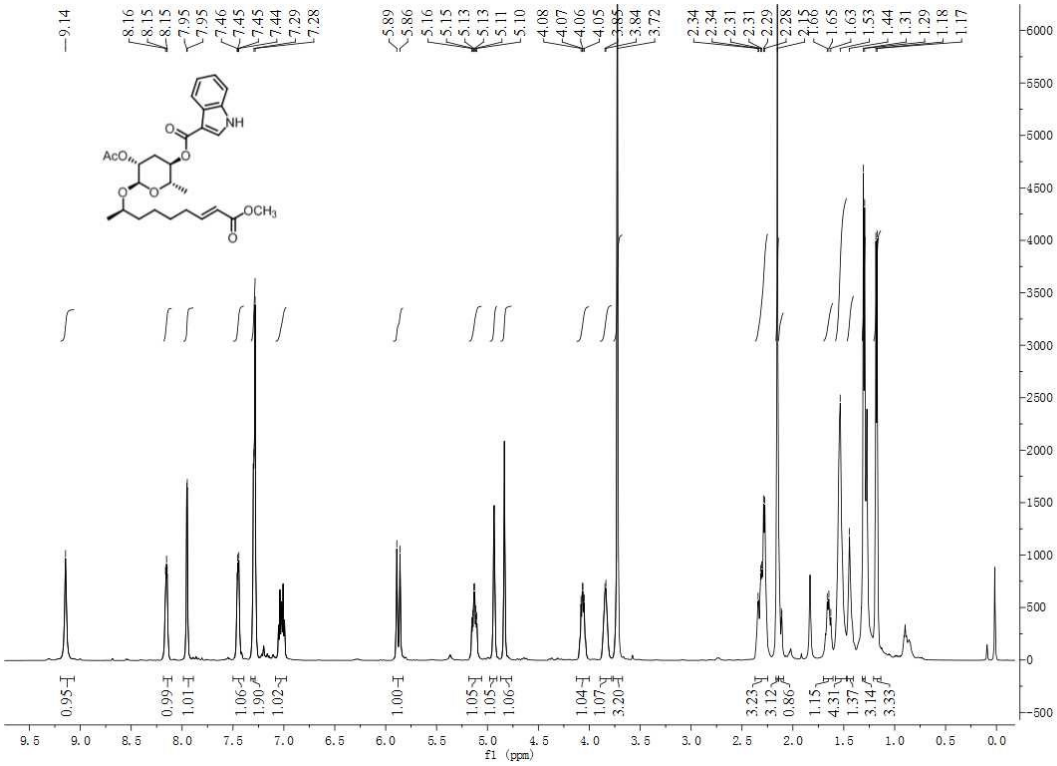


Figure S69. The ^{13}C NMR spectra copy of compound 17a in $CDCl_3$ (126 MHz)

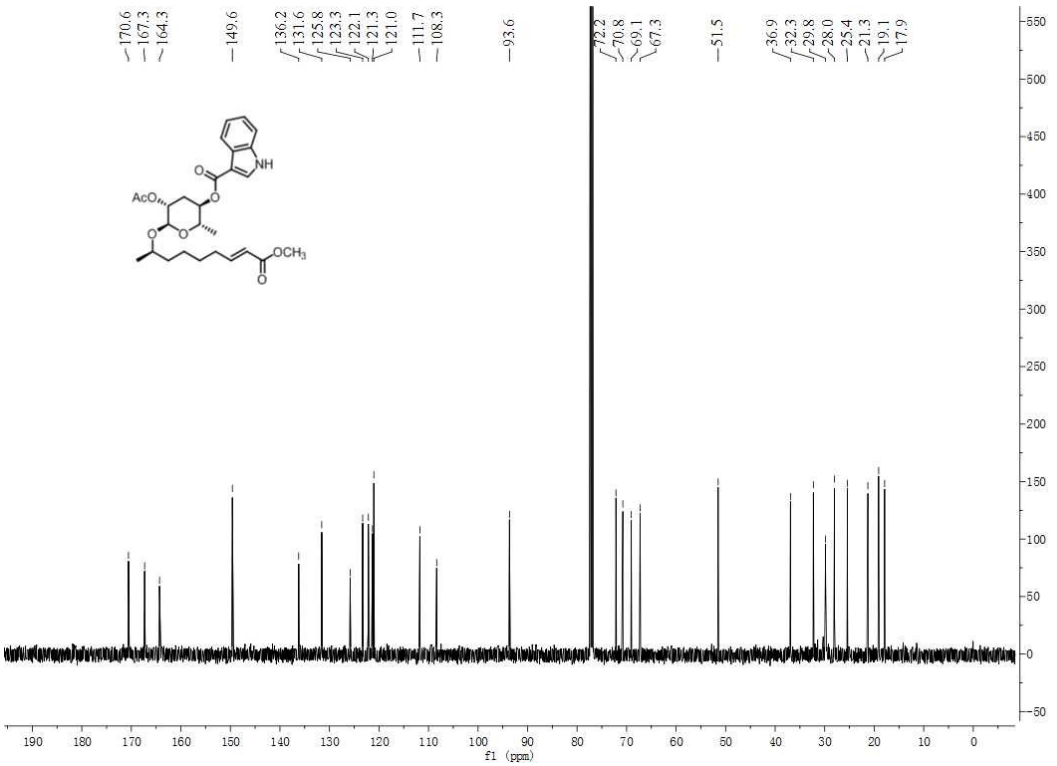


Figure S70. The ^{1}H NMR spectra copy of compound 18a in CD_3OD (400 MHz)

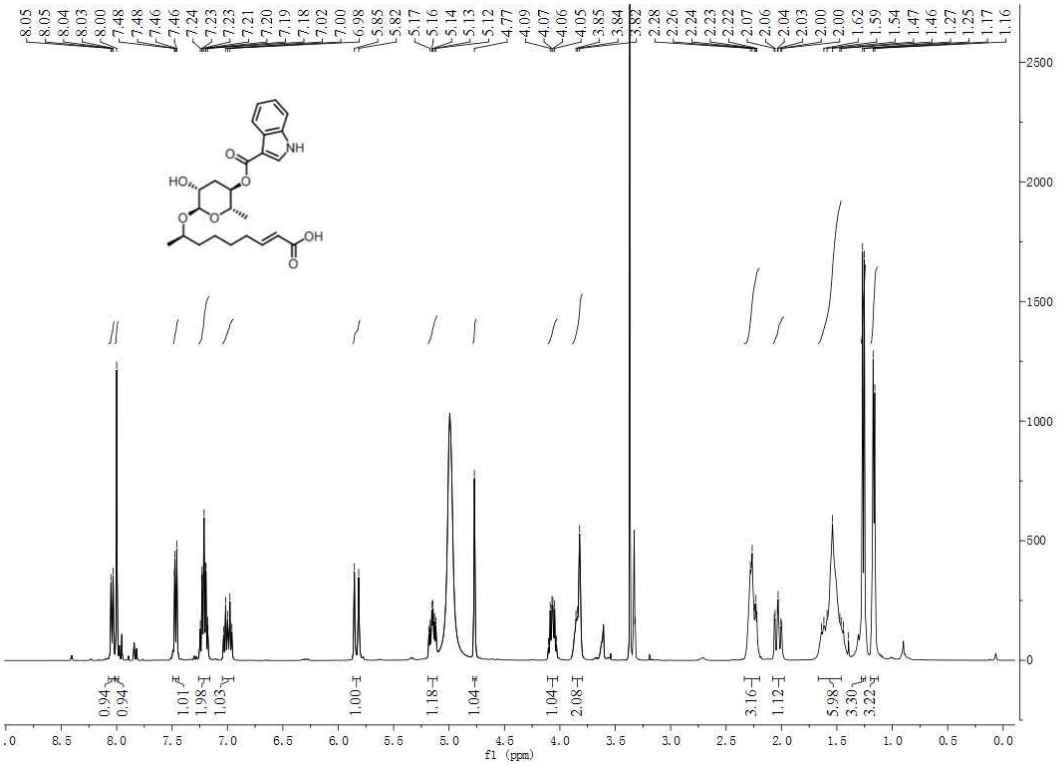


Figure S71. The ^{13}C NMR spectra copy of compound 18a in CD_3OD (101 MHz)

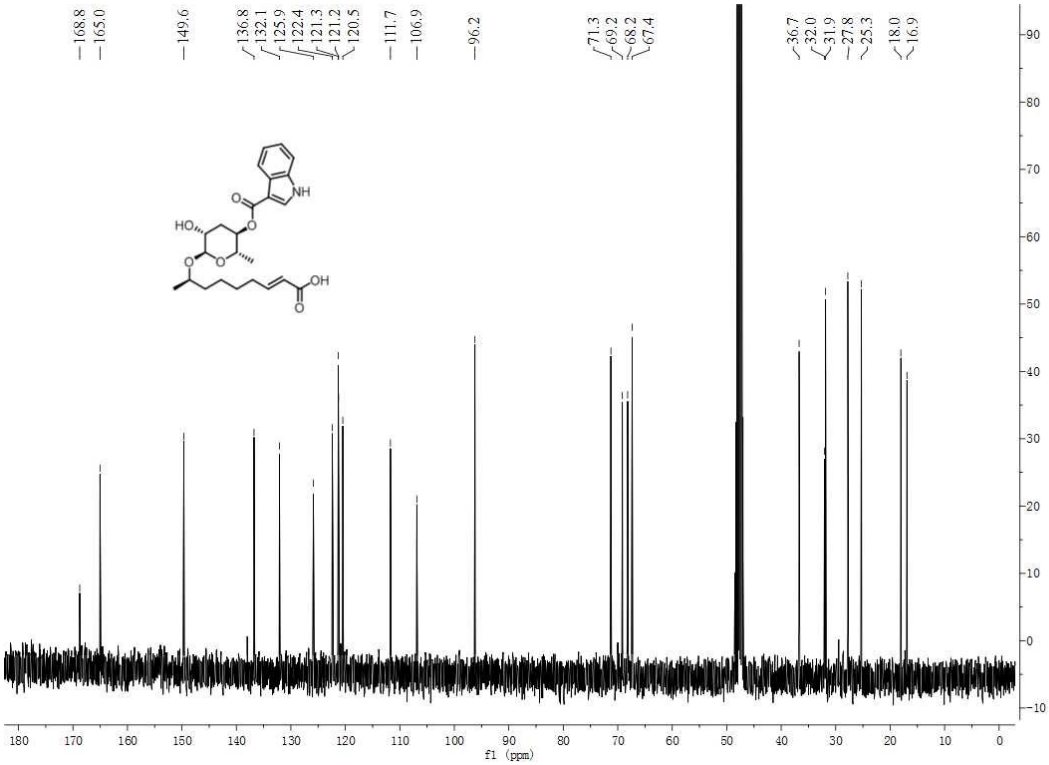


Figure S72. The ^{1}H NMR spectra copy of compound 16b in $CDCl_3$ (500 MHz)

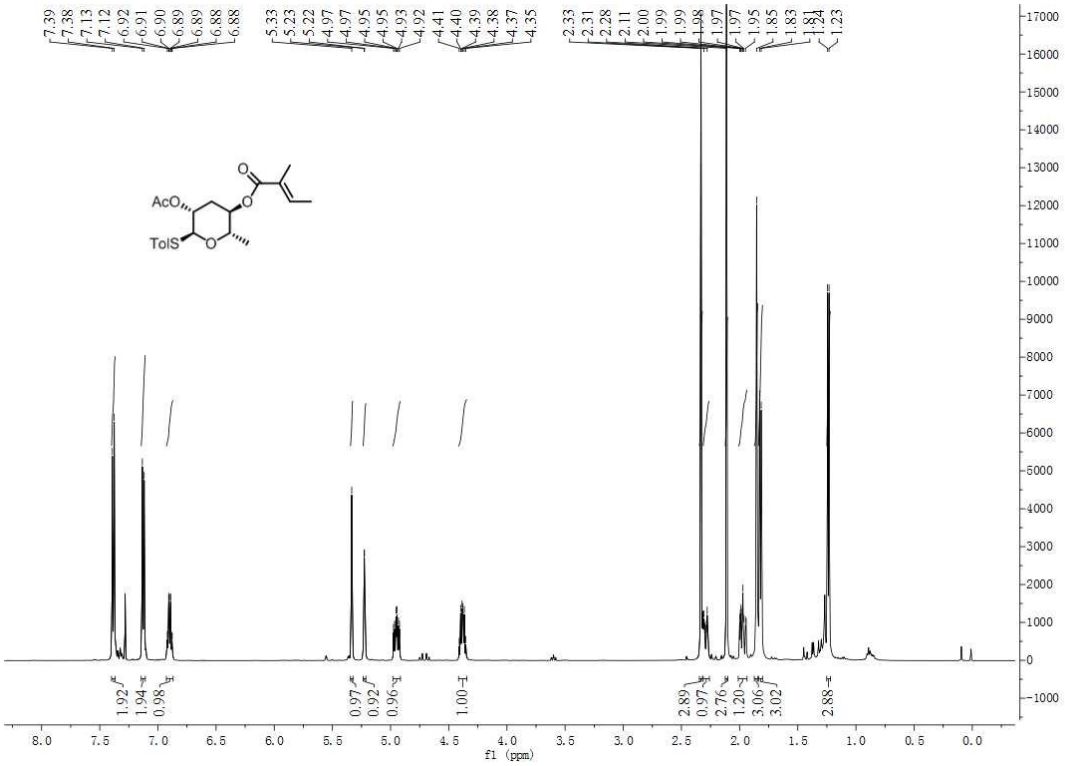


Figure S73. The ^{13}C NMR spectra copy of compound 16b in $CDCl_3$ (126 MHz)

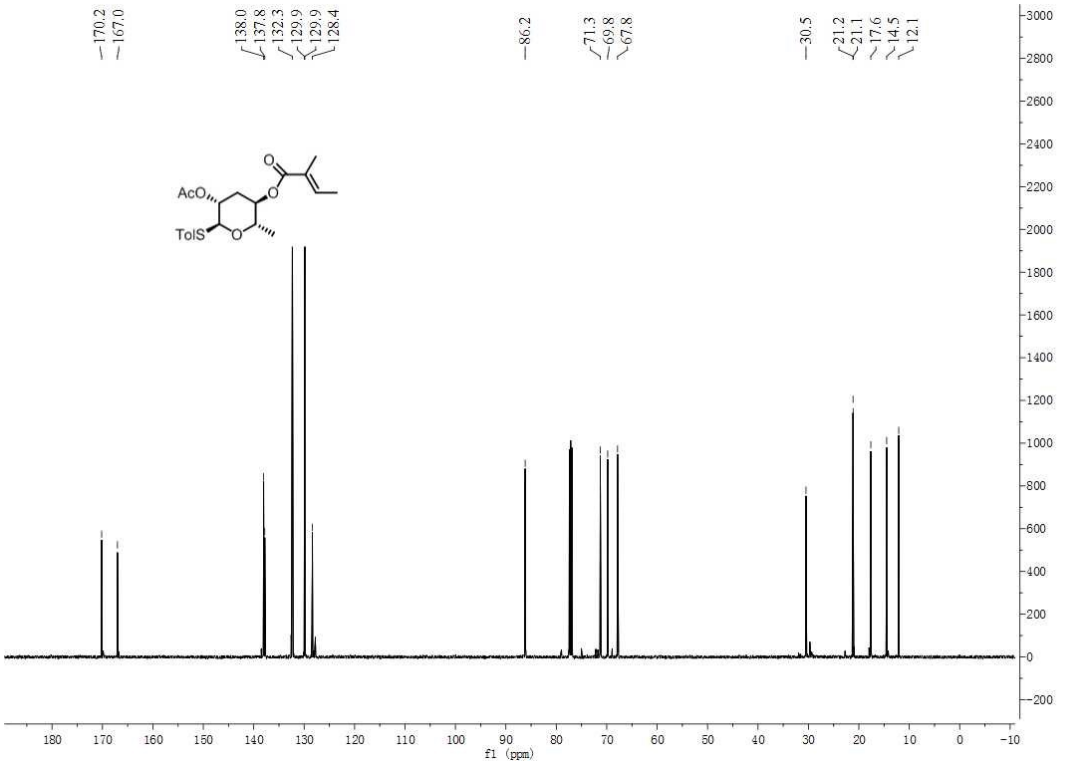


Figure S74. The ^{1}H NMR spectra copy of compound 17b in $CDCl_3$ (400 MHz)

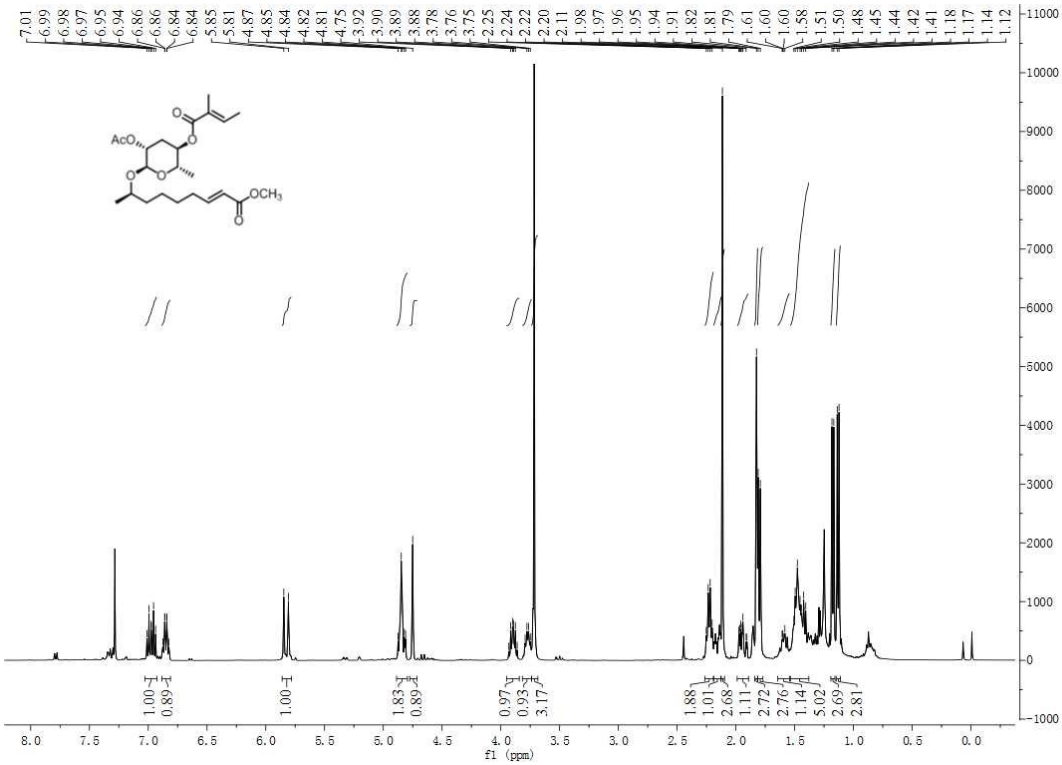


Figure S75. The ^{13}C NMR spectra copy of compound 17b in $CDCl_3$ (101MHz)

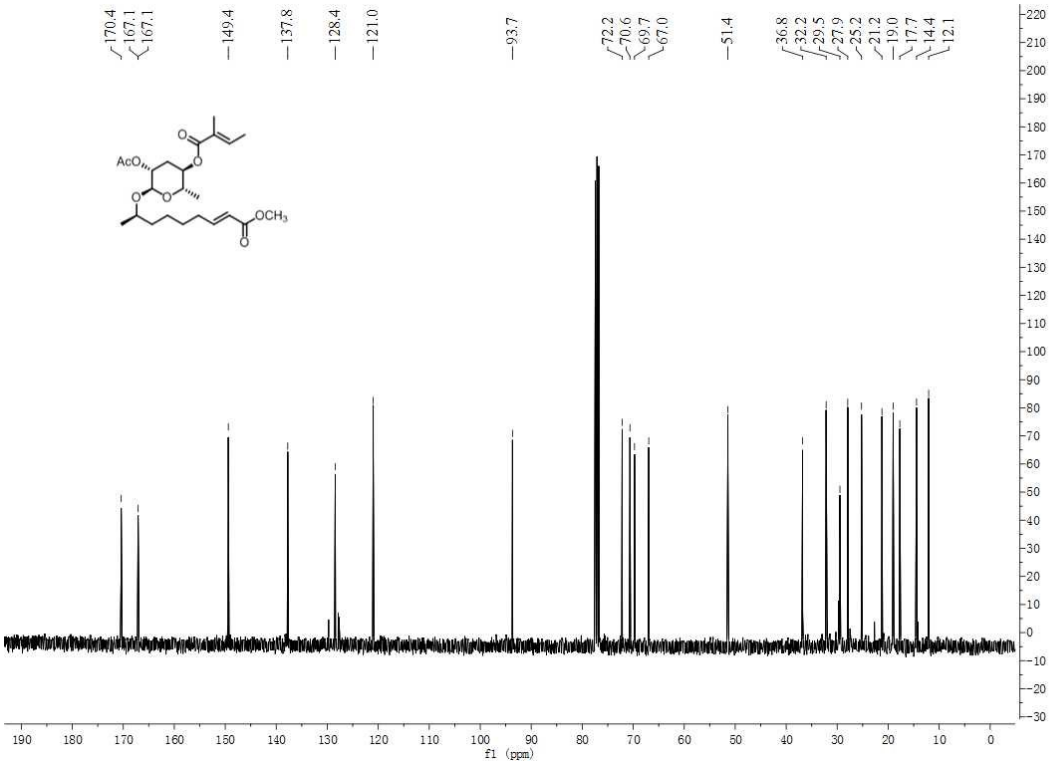


Figure S76. The ^{1}H NMR spectra copy of compound 18b in $CDCl_3$ (400 MHz)

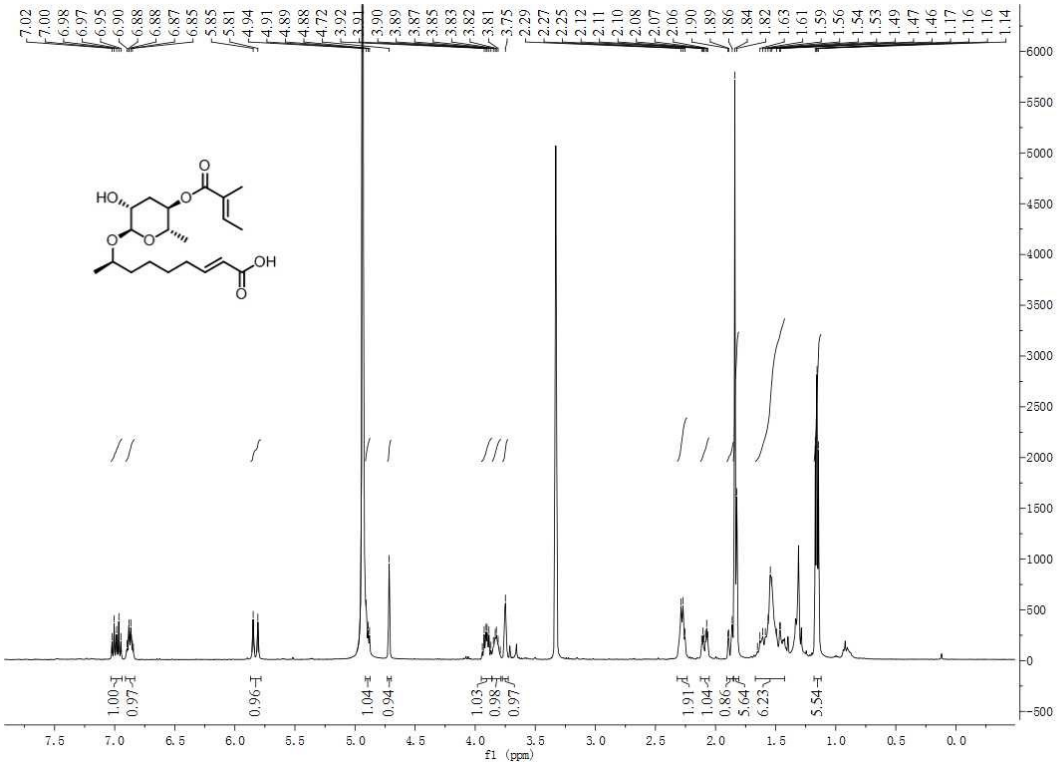
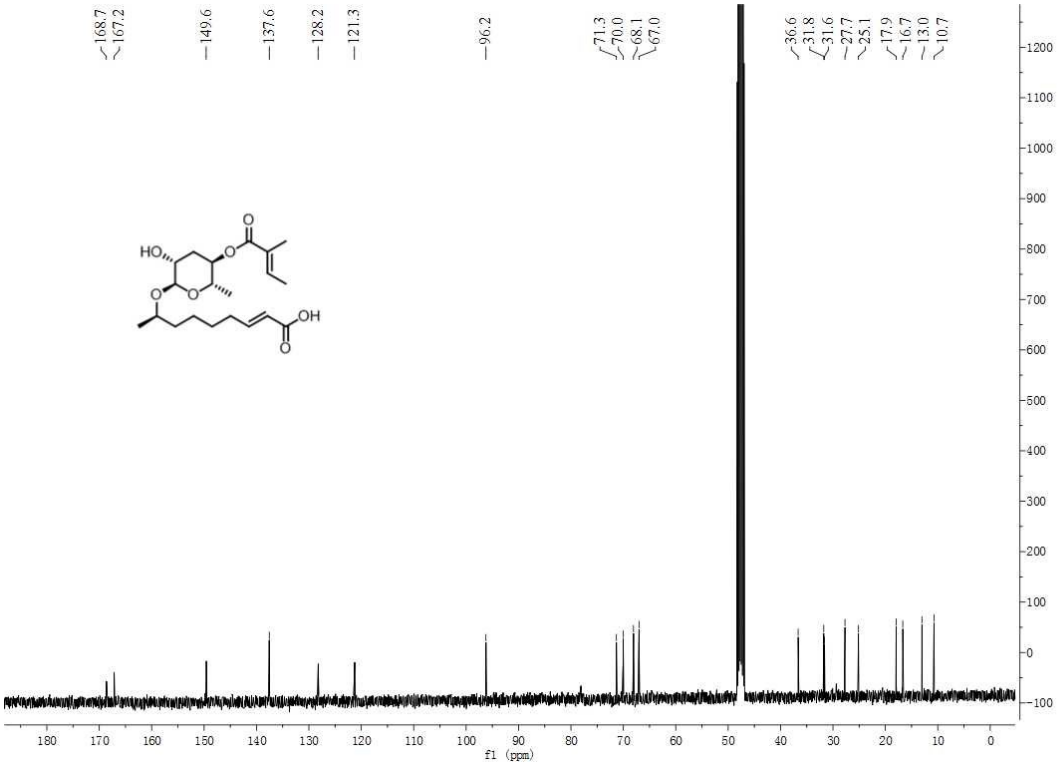


Figure S77. The ^{13}C NMR spectra copy of compound 18b in $CDCl_3$ (101 MHz)



References

1. Miura, K.; Tsukagoshi, T.; Hirano, T.; Nishio, T.; Hakamata, W., Development of Fluorogenic Substrates of α-L-Fucosidase Useful for Inhibitor Screening and Gene-expression Profiling. *ACS Med. Chem. Lett.* **2019,** *10* (9), 1309-1313.
2. Jeong, P.-Y.; Jung, M.; Yim, Y.-H.; Kim, H.; Park, M.; Hong, E.; Lee, W.; Kim, Y. H.; Kim, K.; Paik, Y.-K., Chemical structure and biological activity of the Caenorhabditis elegans dauer-inducing pheromone. *Nature* **2005,** *433* (7025), 541-545.
3. Tai, C.-A.; Kulkarni, S. S.; Hung, S.-C., Facile Cu(OTf)2-Catalyzed Preparation of Per-O-acetylated Hexopyranoses with Stoichiometric Acetic Anhydride and Sequential One-Pot Anomeric Substitution to Thioglycosides under Solvent-Free Conditions. *J. Org. Chem.* **2003,** *68* (22), 8719-8722.
4. Fouque, E.; Rousseau, G., Enzymic resolution of medium-ring lactones. Synthesis of (S)-(+)-phoracantholide I. *Synthesis* **1989,** (9), 661-6.
5. Mochizuki, N.; Yamada, H.; Sugai, T.; Ohta, H., A synthesis of (R)-recifeiolide by the aid of a biochemical reaction as the key-step. *Bioorg. Med. Chem.* **1993,** *1* (1), 71-5.
6. Zhang, Y. K.; Sanchez-Ayala, M. A.; Sternberg, P. W.; Srinivasan, J.; Schroeder, F. C., Improved Synthesis for Modular Ascarosides Uncovers Biological Activity. *Org. Lett.* **2017,** *19* (11), 2837-2840.
7. Hollister, K. A.; Conner, E. S.; Zhang, X.; Spell, M.; Bernard, G. M.; Patel, P.; de Carvalho, A. C. G. V.; Butcher, R. A.; Ragains, J. R., Ascaroside activity in Caenorhabditis elegans is highly dependent on chemical structure. *Bioorg. Med. Chem.* **2013,** *21* (18), 5754-5769.
8. Noguez, J. H.; Conner, E. S.; Zhou, Y.; Ciche, T. A.; Ragains, J. R.; Butcher, R. A., A Novel Ascaroside Controls the Parasitic Life Cycle of the Entomopathogenic Nematode Heterorhabditis bacteriophora. *ACS Chem. Biol.* **2012,** *7* (6), 961-966.
9. Butcher, R. A.; Ragains, J. R.; Kim, E.; Clardy, J., A potent dauer pheromone component in Caenorhabditis elegans that acts synergistically with other components. *Proc. Natl. Acad. Sci. USA* **2008,** *105* (38), 14288-14292.