Supplementary Material (ESI) for Organic and Biomolecular Chemistry

Design, Synthesis and *in Vitro* Splicing Inhibition of Desmethyl and Carba-Derivatives of Herboxidiene

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Experimental Section

All reactions were carried out under an inert atmosphere, either N_2 or Ar, using magnetic stirring and oven-dried glassware. All solvents were anhydrous and distilled prior to use. Dichloromethane and triethylamine were distilled from calcium hydride. Tetrahydrofuran, diethyl ether, and benzene were distilled from sodium/benzophenone. All other solvents were HPLC grade or better. Flash column chromatography was performed using EM Science 60-200 mesh silica gel. Thin-layer chromatography was performed using 60 F-254 E. Merck silica gel plates. ^{1}H- and ^{13}C-NMR were recorded using Bruker AV-400 MHz, Avance DRX-500, Varian Mercury-Vx-300, and Gemini-2300 spectrometers and use Me_4Si as an internal standard. Optical rotations were recorded on a Perkin-Elmer 341 polarimeter. A Thermo Finnigan LCQ Classic mass was used for MS analyses. The purity of test compounds was determined by HRMS and HPLC analysis. All test compounds showed ≥95% purity.

(1R,3S)-3-acetylcyclohexane-1-carboxylic acid (22): To a stirred solution of $ZnBr_2$ (877 mg, 3.8 mmol) in THF (10 mL) and Et_2O (10 mL) was added dropwise a solution of MeMgBr in THF (1.3 mL, 3 M, 3.9 mmol) at 0 °C over 5 min under argon and left to stir at the same temperature. After 30 min, the mixture was stirred for 1 h at 23 °C, and stopped stirring, settled to precipitate for 1 h.

To a stirred solution of $[Rh(nbd)Cl]_2$ (13 mg, 0.028 mmol) and (*S*)-*tert*-butyphosphino-oxazoline (23 mg, 0.059 mmol) in THF (5 mL) in a separate flask was added the above pre-prepared methylzinc bromide solution (16 mL, decanted from the precipitate via syringe) and a solution of **18** (300 mg, 1.94 mmol) in THF (5 mL) at 0 °C. The mixture was stirred at 23 °C for 30 hrs, quenched by 1 M HCl (10 ml), diluted by H_2O (15 mL), extracted by Et_2O (3 × 20 mL), dried over anhydrous MgSO4, filtered and concentration. The residue was purified over silica gel chromatography (Hexane : Ethyl acetate = 2 : 1) to give compound **23** (160 mg, 48%, 91% ee) as a colorless oil. Chiral HPLC analysis was performed using a chiralcel OD-H column eluting with Hexane : i-PrOH = 99 : 1 0.5 mL/min. $[\alpha]^{20}_D$ = -3.8 (c 1.00, CH_2Cl_2).

Methyl 2-((1R,3S)-3-acetylcyclohexyl)acetate (24): To a stirred solution of **23** (40 mg, 0.23 mmol) in THF (4 mL) was added Et_3N (196 µL, 1.4 mmol) and MsCl (54 µL, 0.69 mmol) at 0 °C. The mixture was stirred at the same temperature for 30 min. A fresh prepared solution of CH_2N_2 in Et_2O (excess, 4 mL) was added to the mixture at 0 °C. The mixture was quenched by drops of acetic acid, diluted by H_2O (5 mL), extracted by Et_2O (3 × 10 mL), dried over anhydrous MgSO4, filtered and concentration. The residue was purified over silica gel chromatography (Hexane : Ethyl acetate : Et_3N= 2 : 1: 0.001) to give a colorless oil (35 mg, 78%) which should be used for the next step immediately.

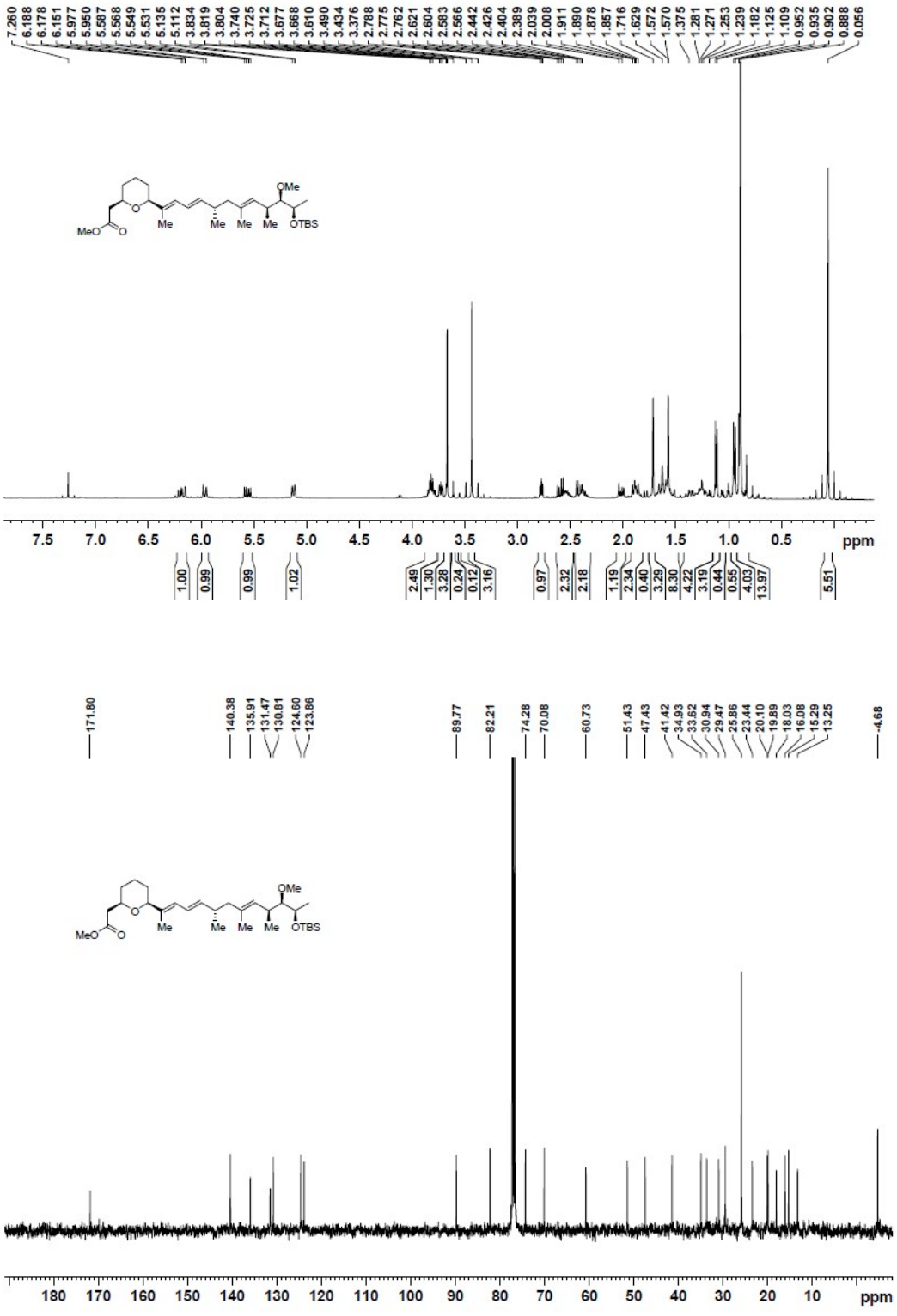
To a stirred solution of above oil (35 mg, 0.18 mmol) in anhydrous MeOH was added a solution of silver benzoate (8.2 mg, 0.03 mmol) in dry Et_3N (100 µL, 0.71 mmol) at 23 °C under argon. The mixture was stirred for 1h and concentrated. The residue was purified over silica gel chromatography (Hexane : Ethyl acetate = 7 : 1) to give compound **24** (21 mg, 59%) as a colorless oil. $[\alpha]^{20}_D$ = +8.3 (c 1.00, CH_2Cl_2); HRMS-ESI (m/z) calc. for $C_{11}H_{18}NaO_3$ $[M + Na]^+$ 221.1154, found 221.1147.

Methyl 2-((1R,3S)-3-((E)-1-iodoprop-1-en-2-yl)cyclohexyl)acetate (25): To a mixture of $CrCl_2$ (61 mg, 0.5 mmol) in THF (2 mL) was added dropwise the solution of the ketone **24** (10 mg, 0.05 mmol) and CHI_3 (59 mg, 0.15 mmol) in THF (2 mL). After being stirred at 23 °C under argon for 4 h, the mixture was quenched by water and extracted with EtOAc. The combined organic phase was washed with water, brine, dried over anhydrous Mg_2SO_4 and concentrated. The residue was purified column chromatography (hexane/EtOAc, 40:1) to give the vinyl iodide **25** (7 mg, 44%) as colorless oil which should be used for the next step immediately.

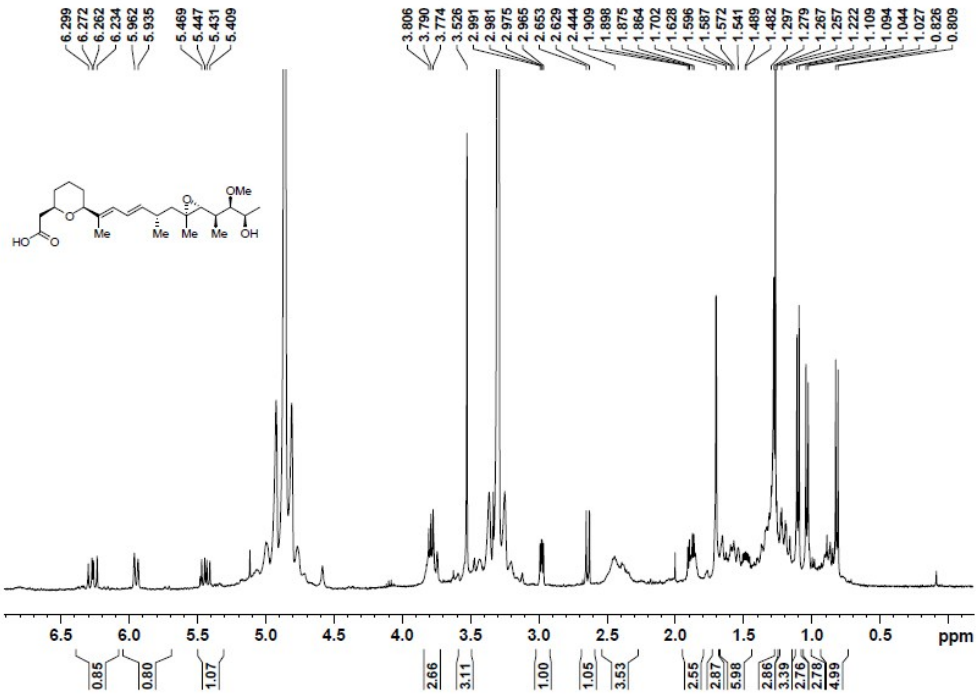
Methyl 2-((1R,3S)-3-((2E,4E,6S,8E,10S,11R,12R)-12-hydroxy-11-methoxy-6,8,10-trimethyltrideca-2,4,8-trien-2-yl)cyclohexyl)acetate (26): A mixture of vinyl iodide **25** (12 mg, 0.037 mmol), boronate **7** (16 mg, 0.034 mmol), $Pd(PPh_3)_4$ (3 mg, 0.002 mmol) and Cs_2CO_3 (220 mg, 0.67 mmol) in THF (2 mL) was stirred at 55 °C under argon for 4 h. It was quenched with water, and then extracted with ether. The combined organic phase was washed with water, brine, dried (Na_2SO_4) and concentrated. The residue was purified column chromatography (hexane/EtOAc, 30:1) to give a colorless oil (13 mg, 72%).

To a solution of above oil (10 mg, 0.023 mmol) in THF (2 mL) was added 1N HCl in MeOH (0.2 mL) at 23 °C. The mixture was stirred for 1 h at the same temperature, and concentrated. The residue was purified column chromatography (hexane : EtOAc = 4 : 1) to give the de-protected product **26** (6.8 mg, 87%) as a colorless oil. $[\alpha]^{20}_D$ = -19.2 (c 0.25, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) 6.18 (dd, *J* = 10.9, 15.1 Hz, 1H), 5.76 (d, *J* = 10.7 Hz, 1H), 5.46 (dd, *J* = 7.5, 15.1 Hz), 4.96 (d, *J* = 9.5 Hz, 1H), 3.70 (brs, 1H), 3.65 (s, 3H), 3.50 (s, 3H), 2.70 (dd, *J* = 4.0, 6.8 Hz, 1H), 2.74-2.60 (m, 1H), 2.37 (t, *J* = 7.0 Hz, 1H), 2.20 (dd, *J* = 3.1, 7.2 Hz, 2H), 2.01 (dd, *J* = 6.9, 13.4 Hz, 1H), 1.95-1.70 (m, 4H), 1.69 (s, 3H), 1.60 (d, *J* = 3 Hz, 3H), 1.35-1.24 (m, 3H), 1.19 (d, *J* = 6.5 Hz, 3H), 0.95 (d, *J* = 6.8 Hz, 3H), 0.93 (d, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 173.3, 140.8, 138.1, 133.6, 129.4, 124.5, 122.9, 89.6, 67.8, 61.3, 51.3, 47.5, 46.8, 41.9, 37.8, 34.9, 34.7, 34.6, 32.5, 30.9, 25.8, 20.3, 19.9, 16.4, 16.2, 14.7; HRMS-ESI (m/z) calc. for $C_{26}H_{44}NaO_4$ $[M + Na]^+$ 443.3137, found 443.3117.

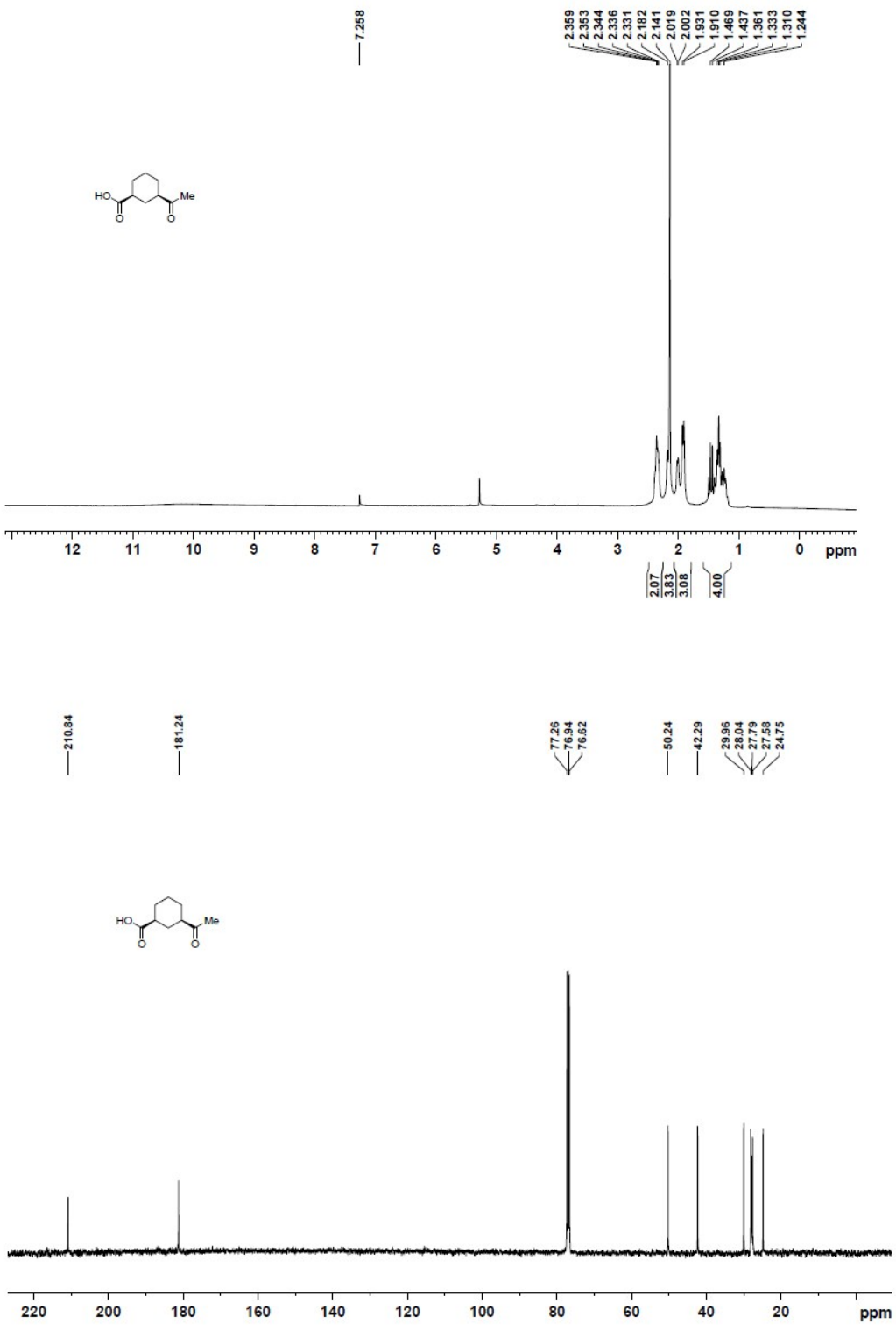
2-((1R,3S)-3-((S,2E,4E)-7-((2R,3R)-3-((2R,3R,4R)-4-hydroxy-3-methoxypentan-2-yl)-2-methyloxiran-2-yl)-6-methylhepta-2,4-dien-2-yl)cyclohexyl) acetic acid (27): To a solution of the **26** (7 mg, 0.016 mmol) and $VO(acac)_2$ (0.88 mg, 0.003 mmol) in CH_2Cl_2 (1 mL) was added *t*-BuOOH (5.5 M in decane, 13.3 μL, 0.07 mmol) at -78 °C. After being stirred at -15– -20 °C for 48 h, it was quenched with Me_2S and stirred at 23 °C for 30 min. It was concentrated and used for next reaction directly. To a solution of the above crude product in MeOH (1 mL) and water (0.2 mL) was added K_2CO_3 (14.7 mg, 0.11 mmol). After being refluxed for 2 h, it was cooled, treated with aq. $NaHSO_4$ (0.01 M, 50 mL) and extracted with EtOAc. The combined organic phase was washed with brine, dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (CH_2Cl_2 : MeOH = 15 : 1) to give the acid **27** (2.8 mg, 40% for 2 steps) as a semi solid. $[\alpha]^{20}_D$ = -15.0 (c 0.10, CH_2Cl_2); ^{1}H NMR (500 MHz, CD_3OD) 6.24 (dd, *J* = 10.5, 15.0 Hz, 1H), 5.74 (d, *J* = 11.0 Hz, 1H), 5.33 (dd, *J* = 9.0, 14.5 Hz, 1H), 3.77 (t, *J* = 6.4 Hz, 1H), 3.51 (s, 3H), 2.96 (dd, *J* = 4.0, 6.4 Hz, 1H), 2.62 (d, *J* = 9.4 Hz, 1H), 2.40 (brs, 1H), 2.15 (t, *J* = 6.5 Hz, 1H), 1.95-1.88 (m, 2H), 1.80-1.73 (m, 3H), 1.67 (s, 3H), 1.47-1.44 (m, 1H), 1.40-1.30 (m, 1H), 1.17-1.10 (m, 2H), 1.08 (d, *J* = 6.8 Hz, 3H), 1.01 (d, *J* = 6.6 Hz, 1H), 0.95-0.87 (m, 2H), 0.80 (d, *J* = 6.8 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) 175.4, 140.7, 136.6, 125.7, 122.8, 86.9, 68.4, 66.4, 61.2, 60.3, 41.6, 37.7, 34.9, 34.8, 34.7, 32.2, 30.8, 25.6, 21.1, 18.2, 15.2, 13.4, 9.6; HRMS-ESI (m/z) calc. for $C_{25}H_{42}NaO_5$ $[M + Na]^+$ 445.2930, found 445.2913.



NMR Spectra for compound 17



NMR Spectra for compound 5



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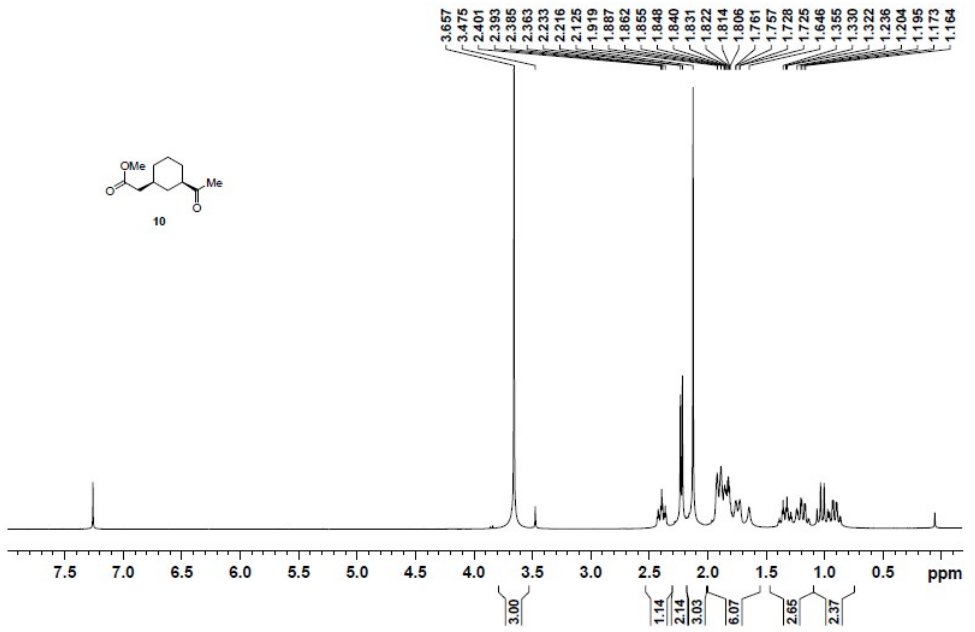

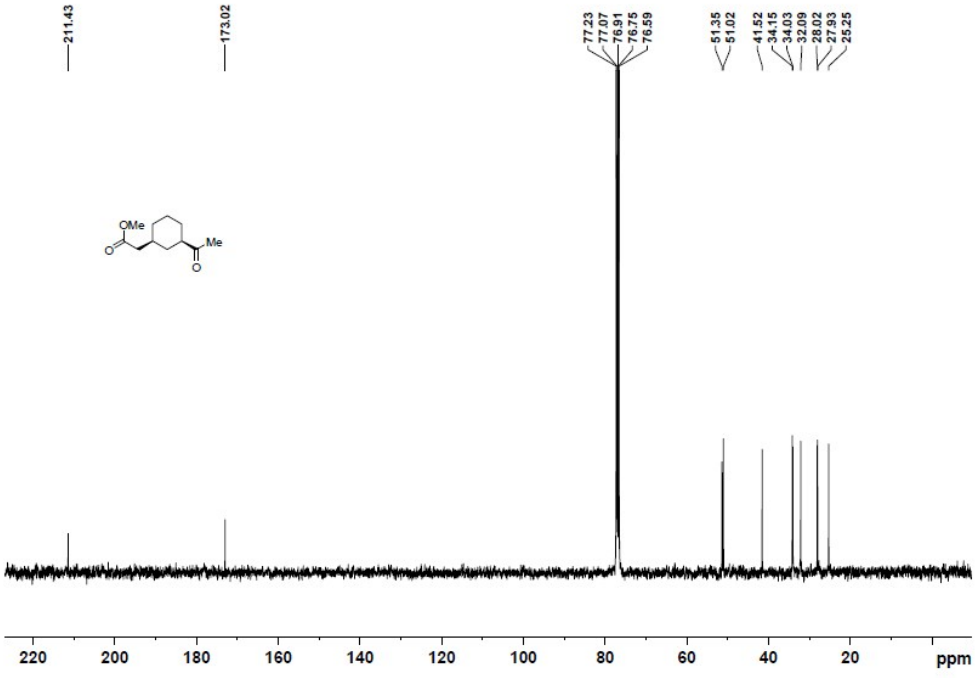

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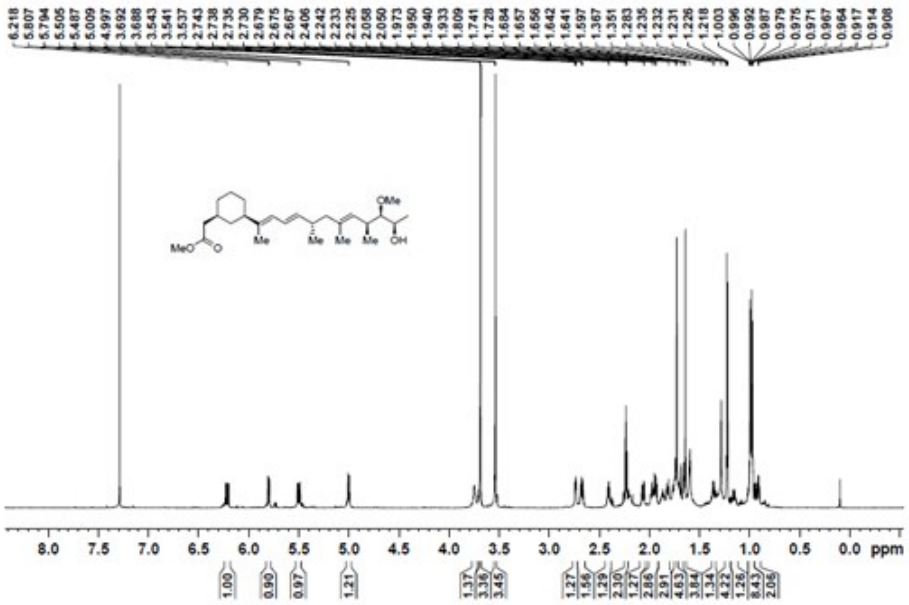

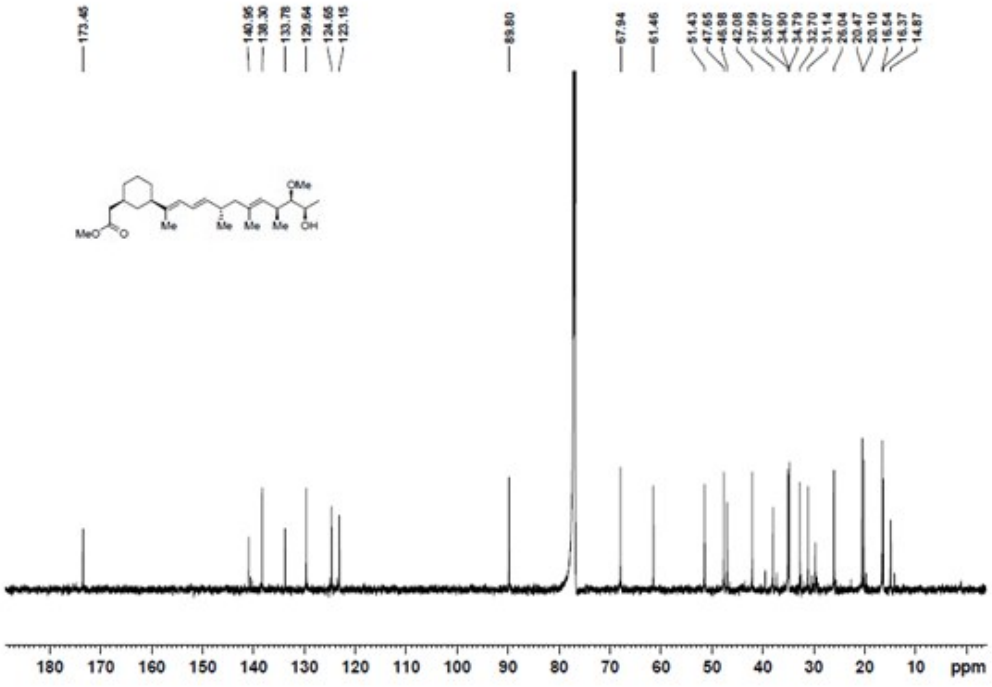

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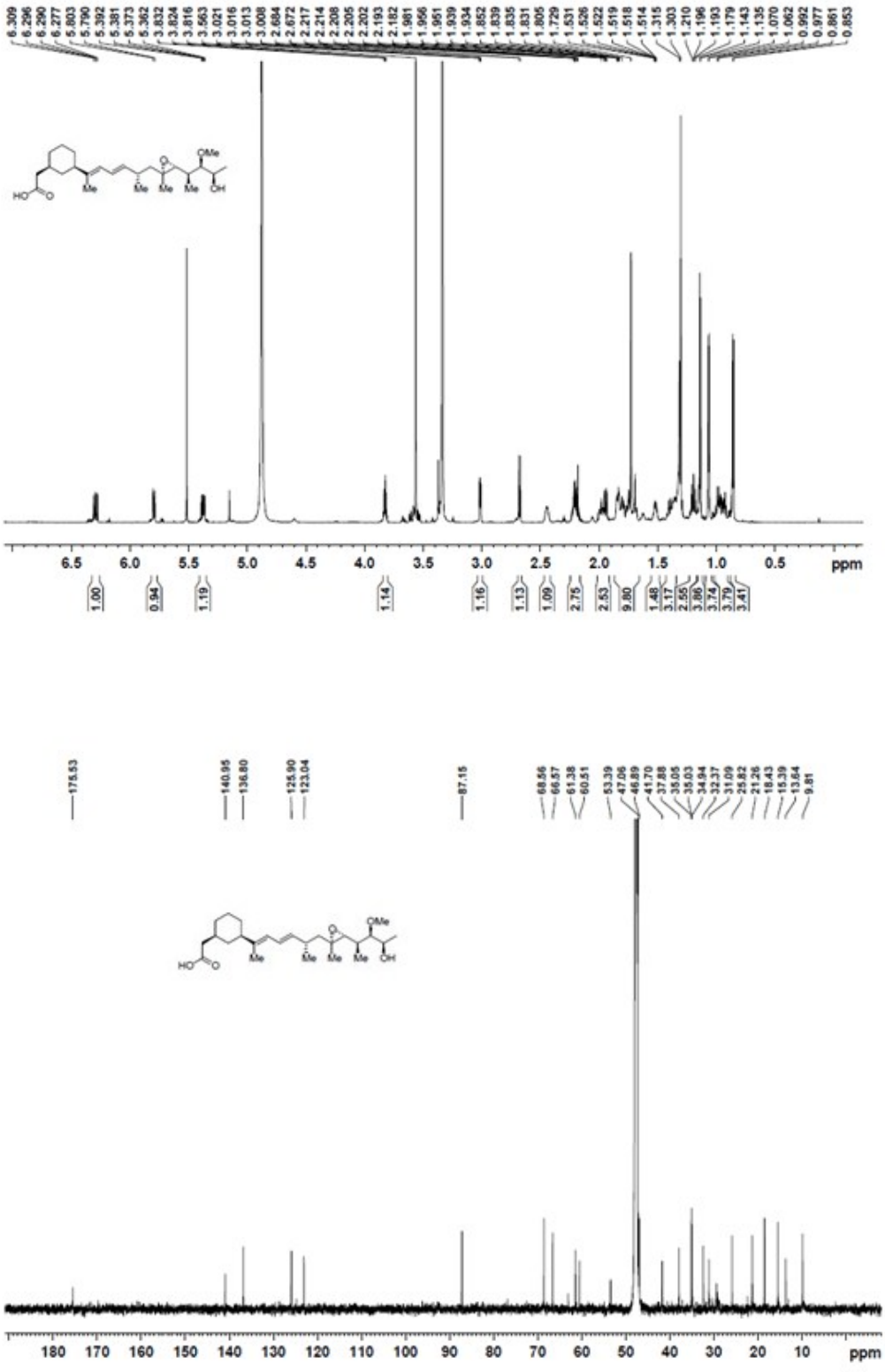

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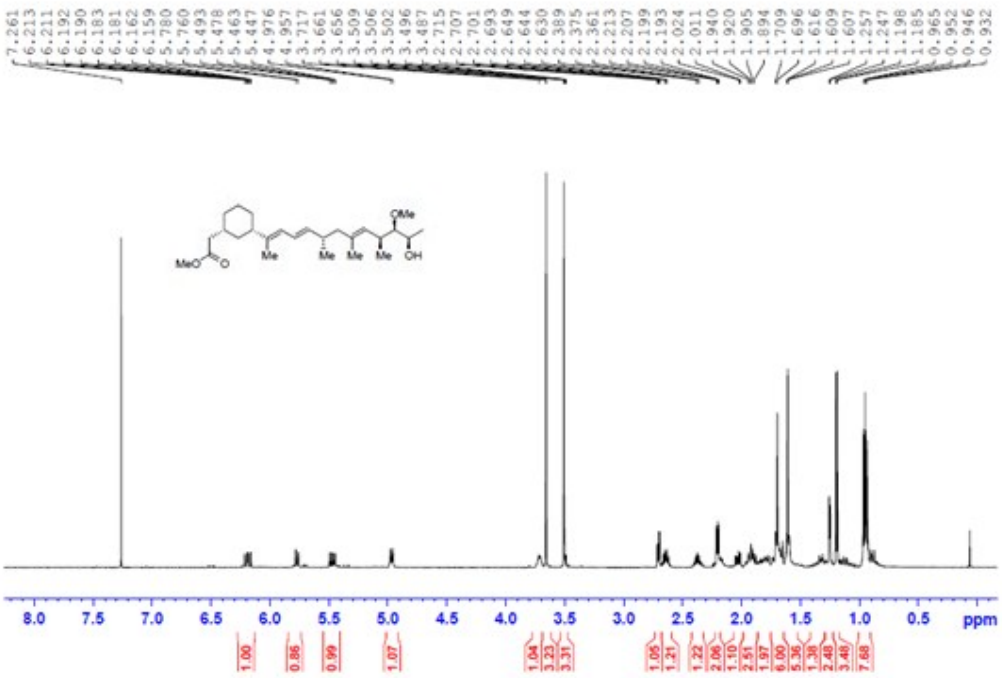

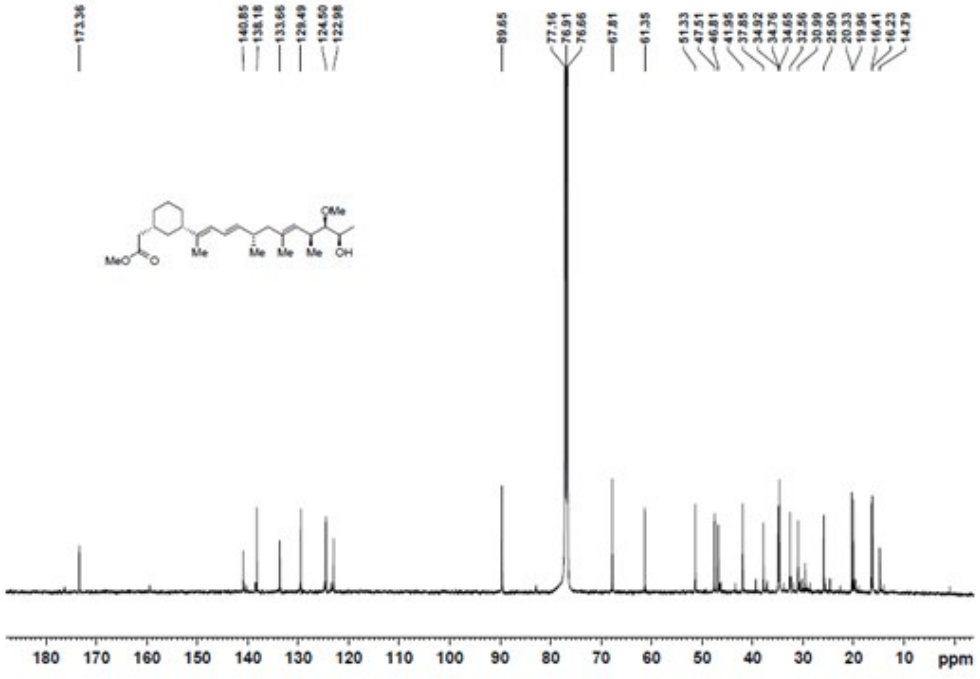

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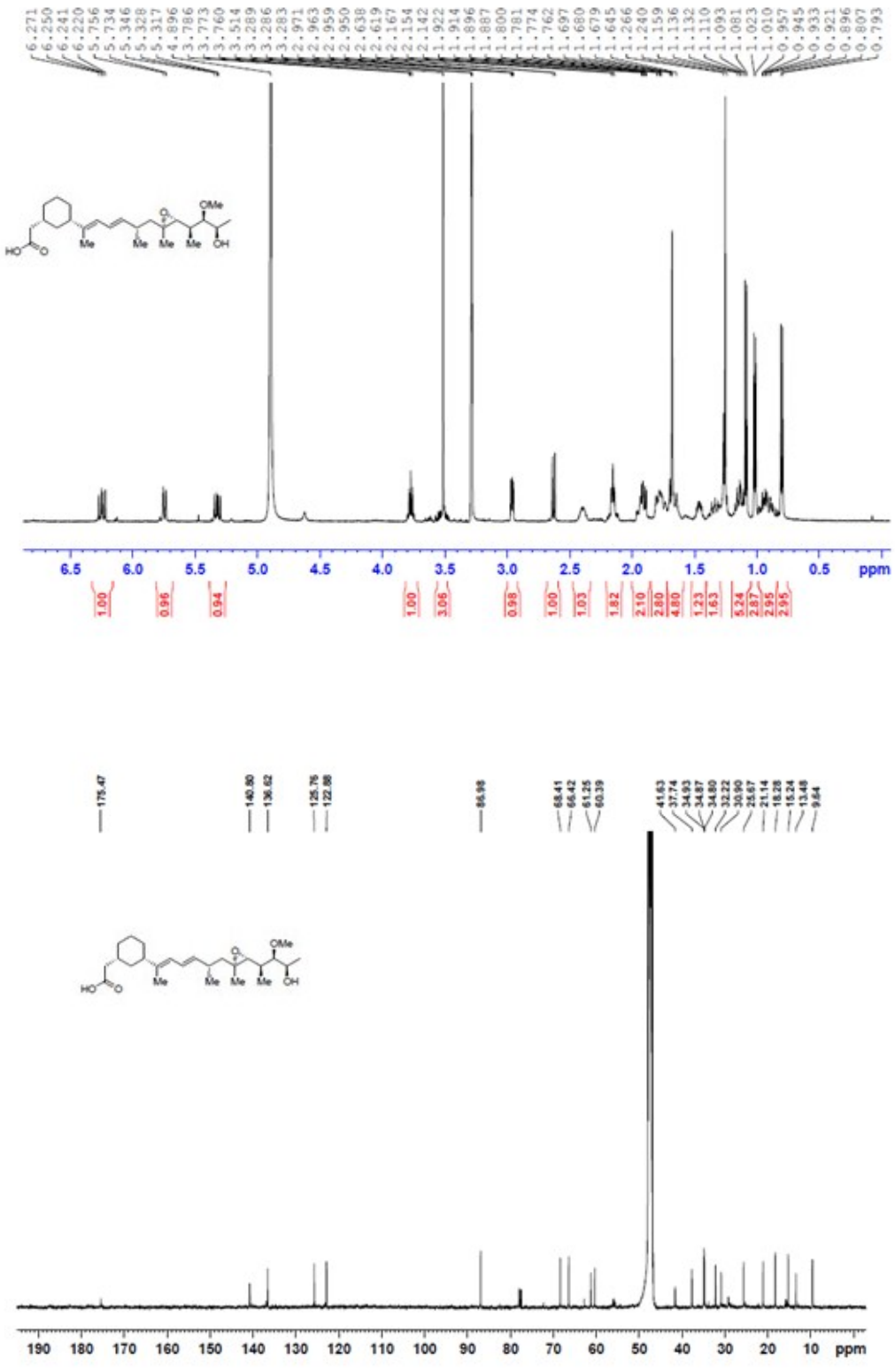

NMR Spectra for compound 27