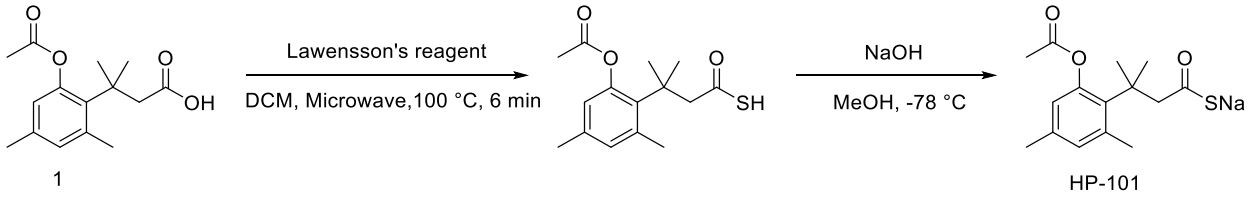
General Information.

All reagents and solvents were of reagent grade and were purchased from Aldrich. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded on a Bruker Avance 400 MHz NMR spectrometer. Mass spectral analyses were performed on an ABI API 3200 (ESI-Triple Quadruple). HPLC was performed on a Shimadzu Prominence UFLC (column: Waters C18 3.5 μM, 4.6×100 mm). UV-Vis absorption spectra were recorded on a Shimadzu PharmaSpec UV-1700 UV-Visible spectrophotometer. Fluorescence spectra were recorded on a Shimadzu RF-5301PC fluorometer. 96-Well plates were read and recorded on a PerkinElmer 1420 multi-label counter. Compounds **1,**[1] **2**,[1a] **8,**[1a] WSP-5[2] and GYY 4137[3] were synthesized according to literature procedures.

Synthesis of HPs

Synthesis of "trimethyl lock"-based H_2S prodrug HP-101 (Scheme 1)

Scheme 1



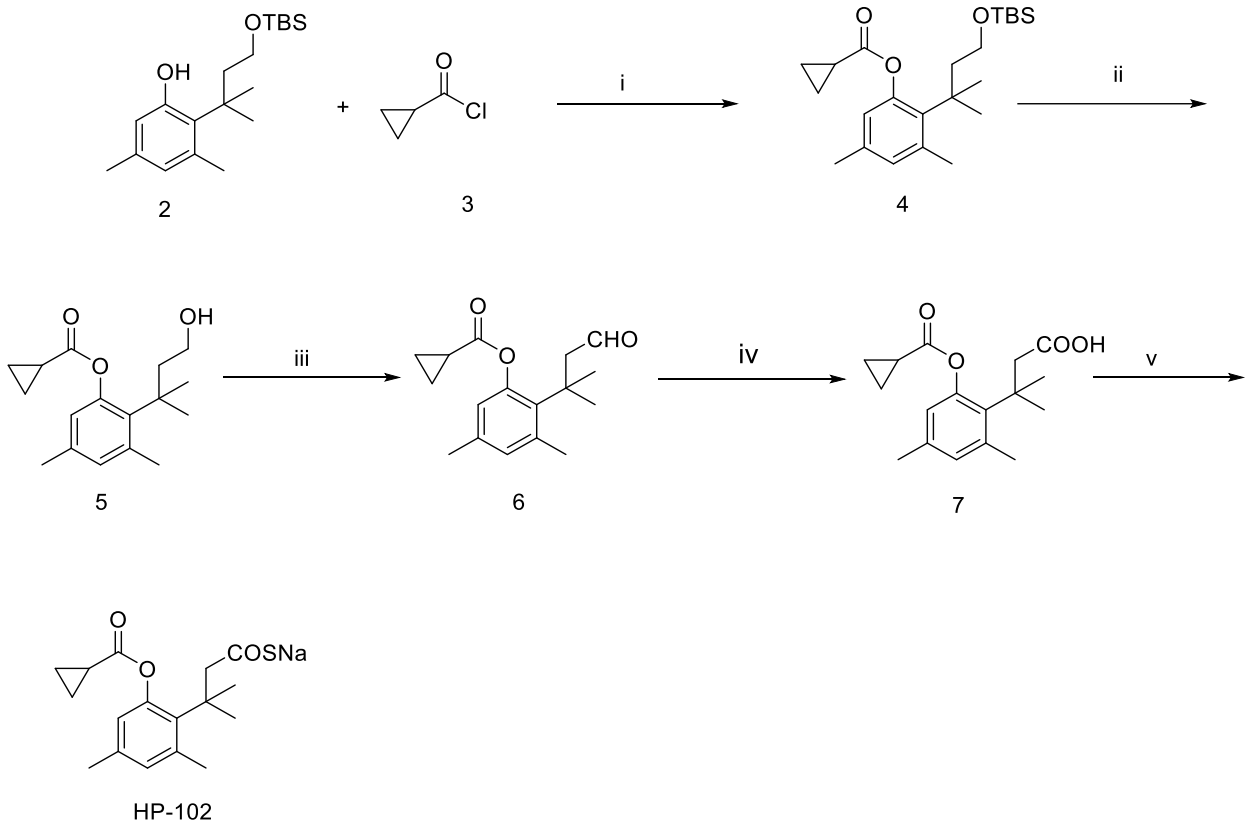
*Synthesis of sodium 3-(2-acetoxy-4, 6-dimethylphenyl)-3-methylbutanethioate (**HP-101**).* A solution of 3-(2-acetoxy-4,6-dimethylphenyl)-3-methylbutanoic acid (**1**, 78 mg, 0.3 mmol), Lawesson's reagent (60 mg, 0.15 mmol) and 1.5 mL CH_2Cl_2 in a sealed tube was subjected to microwave irradiation (100 ℃, 6 min). After completion of reaction, the solution mixture was diluted with dichloromethane (DCM, 5 mL). The organic layer was washed by 1 N HCl and brine, and dried over anhydrous sodium sulfate. Then, after filtration, DCM was removed under vacuum. The residue was purified by flash column chromatography (hexane: ethyl acetate =10:1) to give an oily residue (59 mg). The oil product was dissolved in methanol, and a NaOH solution (8.4 mg in 2 mL methanol) was added at -78 ℃. After 3 minutes, the methanol in the reaction mixture was removed by rotavapor. Diethyl ether was added into the crude product, and the final

product was precipitated from diethyl ether as a white solid (57 mg, 67%). ^{1}H NMR (CD_3OD): δ 6.80 (s, 1H, Ph-H), 6.53 (s, 1H, Ph-H), 3.33 (s, -CH_2-CO-), 2.58 (s, 3H, Ph-CH_3), 2.32 (s, 3H, -CO-CH_3), 2.21 (s, 3H, Ph-CH_3), 1.54 (s, 6H, Ph-C(CH_3)$_2$-); ^{13}C NMR (CD_3OD): δ219.0, 172.4, 150.7, 139.4, 137.0, 136.4, 132.9, 123.9, 64.4, 40.6, 31.8, 25.7, 22.0, 20.2. HRMS calcd for $C_{15}H_{19}O_3S$ [M-H]$^-$ 279.1055, found: 279.1051.

Synthesis of HP-102 (

Scheme 1.)

Scheme 1.



Reagents and conditions (i) Et_3N, DCM, 0 ℃-rt, 12 h, 46%; (ii) AcOH/H_2O, THF, rt, 12 h, 91%; (iii) Pyridinium chlorochromate (PCC), DCM, rt, 2 h, 83%; (iv) $NaClO_2$/NaH_2PO_4, 2-methylbut-2-ene, *t*-BuOH, rt, 2 h, 52%; (v) 1) Lawesson's reagent, DCM, microwave, 6 min; 2) NaOH, methanol, -78 ℃, 56% for the last two steps .

[0001] *Synthesis of 2-(4-((tert-butyldimethylsilyl)oxy)-2-methylbutan-2-yl)-3,5-dimethylphenyl cyclopropanecarboxylate* (**4**). To a solution of 2-(4-((tert-butyldimethylsilyl)oxy)-2-methylbutan-2-yl)-3,5-dimethylphenol (**2**, 1.9 g, 5.9 mmol) and Et_3N (1.2 ml, 8.8 mmol) in DCM (150 mL) was added dropwise cyclopropanecarbonyl chloride (**3**, 0.8 ml, 8.8 mmol) at 0 ℃ during a period of 10 min. The mixture was allowed to warm to room temperature and was stirred for an additional 12 h. Then the reaction was quenched with the addition of H_2O (100 mL), and extracted with ethyl acetate (2 × 150 mL). The combined organic phase was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to give the crude product, which was purified by column chromatography (hexane: ethyl acetate =100:1) to give a colorless oil (1.04 g, 46%). ^{1}H NMR ($CDCl_3$): δ6.79 (s, 1H), 6.56 (s, 1H), 3.50 (t, *J* = 8.0Hz, 2H), 2.52 (s, 3H), 2.22 (s, 3H), 2.06 (t, *J* = 8.0Hz, 2H), 1.86-1.80 (m, 1H), 1.49 (s, 6H), 1.17-1.13 (m, 2H), 1.01-0.95 (m, 2H), 0.85 (s, 9H), -0.02 (s, 6H); ^{13}C NMR ($CDCl_3$): 174.1, 150.1, 138.4, 136.0, 134.3, 132.3, 123.2, 61.0, 46.1, 39.3, 32.0, 26.1, 25.4, 20.3, 18.4, 13.7, 8.9, -5.2. HRMS calcd for $C_{23}H_{38}O_3Si$ $[M+H]^+$ 391.2663, found: 391.2649.

Synthesis of 2-(4-hydroxy-2-methylbutan-2-yl)-3,5-dimethylphenyl cyclopropanecarboxylate (**5**). To a solution of 2-(4-((tert-butyldimethylsilyl)oxy)-2-methylbutan-2-yl)-3,5-dimethylphenyl cyclopropanecarboxylate (**4**, 1.04 g, 2.7 mmol) in tetrahydrofuran (THF, 15 mL) was added H_2O (15 mL) and AcOH (45 mL). The reaction mixture was stirred at room temperature for 4 h, quenched with H_2O (50 mL), and extracted with ethyl acetate (2 × 150 mL). The combined organic phase was dried over anhydrous Na_2SO_4, evaporated under reduced pressure, and purified by silica gel column chromatography (hexane: ethyl acetate =6:1) to give a colorless oil (680 mg, 91%). ^{1}H NMR ($CDCl_3$): δ 6.81 (s, 1H), 6.55 (s, 1H), 3.54 (t, *J* = 8.0 Hz, 2H), 2.52 (s, 3H), 2.22 (s, 3H), 2.06 (t, *J* = 8.0 Hz, 2H), 1.87-1.82 (m, 1H), 1.51 (s, 6H), 1.18-1.14 (m, 2H), 1.04-0.99 (m, 2H); ^{13}C NMR ($CDCl_3$): 174.8, 150.0, 138.5, 136.3, 134.1, 132.5, 123.4, 60.7, 45.9, 39.3, 32.2, 25.5, 20.3, 13.7, 9.1. HRMS calcd for $C_{17}H_{24}O_3$ $[M+H]^+$277.1798, found: 277.1789.

Synthesis of 3,5-dimethyl-2-(2-methyl-4-oxobutan-2-yl)phenylcyclopropanecarboxylate (**6**). To a solution of PCC (1.5 g, 7.0 mmol) in DCM (20 mL) was added dropwise 2-(4-hydroxy-2-methylbutan-2-yl)-3,5-dimethylphenyl cyclopropanecarboxylate (**5**, 0.96 g, 3.5 mmol) in DCM (25 mL) at room temperature. After 2 h, the mixture was directly subjected to column

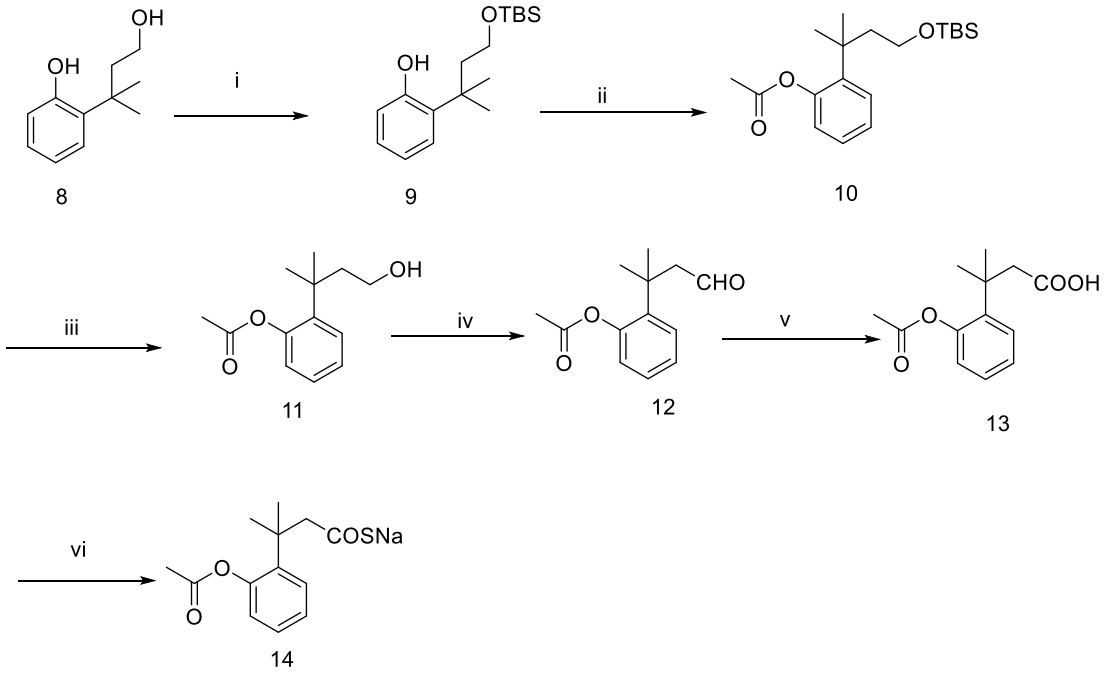
chromatography (hexane: ethyl acetate =10:1) to obtain the pure product as colorless oil (0.8 g, 83%). ^{1}H NMR ($CDCl_3$): δ 9.55 (t, *J* = 4Hz, 1H), 6.84 (s, 1H), 6.61 (s, 1H), 2.84 (d, *J* = 4.0 Hz, 2H), 2.53 (s, 3H), 2.23 (s, 3H), 1.86-1.80 (m, 1H), 1.57 (s, 6H), 1.17-1.14 (m, 2H), 1.05-1.00 (m, 2H); ^{13}C NMR ($CDCl_3$): 203.3, 174.1, 149.7, 137.9, 136.9, 132.8, 132.7, 123.5, 56.8, 38.3, 31.7, 25.5, 20.4, 13.6, 9.1. HRMS calcd for $C_{17}H_{22}O_3$ $[M+H]^+$275.1642, found: 275.1632.

Synthesis of 3-(2-((cyclopropanecarbonyl)oxy)-4,6-dimethylphenyl)-3-methylbutanoic acid (**7**). To a solution of 3,5-dimethyl-2-(2-methyl-4-oxobutan-2-yl)phenyl cyclopropanecarboxylate (**6**, 200 mg, 0.73 mmol) in *t*-BuOH (4 mL) and 2-methylbut-2-ene (0.7 mL), $NaClO_2$ (98 mg, 1.08 mmol) in 0.67 M NaH_2PO_4 (0.8 mL) was added dropwise at room temperature. After 2 h, the reaction mixture was quenched with H_2O (10 mL), and extracted with ethyl acetate (2 × 50 ml). The combined organic phase was dried over anhydrous Na_2SO_4 and then evaporated under reduced pressure to give the crude product, which was purified by column chromatography (hexane: ethyl acetate =5:1) to yield a white solid (110 mg, 52%). ^{1}H NMR ($CDCl_3$): δ 6.80 (s, 1H), 6.59 (s, 1H), 2.86 (s, 2H), 2.53 (s, 3H), 2.22 (s, 3H), 1.89-1.83 (m, 1H), 1.58 (s, 6H), 1.18-1.14 (m, 2H), 1.03-0.98 (m, 2H); ^{13}C NMR ($CDCl_3$): 177.5, 174.2, 149.7, 138.1, 136.4, 133.5, 132.5, 123.2, 47.7, 38.9, 31.4, 25.4, 20.4, 13.6, 9.1. HRMS calcd for $C_{17}H_{22}O_4$ $[M+H]^+$291.1591, found: 291.1580.

Synthesis of Sodium 3-(2-((cyclopropanecarbonyl)oxy)-4,6-dimethylphenyl)-3-methy lbutanethioate (**HP-102**). To a solution of 3-(2-((cyclopropanecarbonyl)oxy)-4,6-dimethylphenyl)-3-methylbutanoic acid (**7**, 110 mg, 0.38 mmol) in DCM (5 mL) was added Lawesson's reagent (77 mg, 0.19 mmol). The mixture was heated in a microwave at 100 ℃ for 6 min. The mixture was directly subjected to column chromatography (hexane: ethyl acetate =25:1) to obtain the pure product as colorless oil, which was then dissolved in 5 ml methanol. Then 2.5 ml of 0.1 M NaOH methanol solution was added at -78 ℃. After 5 min, the mixture was allowed to warm to room temperature and the solvent was removed by vacuum. The final product was achieved by precipitation from ether as a white solid (70 mg, 56%). ^{1}H NMR (400 MHz, CD_3OD): δ 6.77 (d, *J* = 0.8 Hz, 1H), 6.46 (d, *J* = 0.8 Hz, 1H), 3.35 (s, 2H), 2.56 (s, 3H), 2.18 (s, 3H), 1.99-1.98 (m, 1H), 1.53 (s, 6H), 1.09-1.04 (m, 4H); ^{13}C NMR ($CDCl_3$): 196.1, 174.0, 149.8, 138.1, 136.7, 133.0, 132.6, 123.3, 58.5, 39.9, 31.5, 25.6, 20.4, 13.7, 9.2. HRMS calcd for $C_{17}H_{21}NaO_3S$ $[M+H]^+$329.1182, found: 329.1168.

Synthesis of HP-103 (Scheme 3)

Scheme 3.



Reagents and conditions: (i) tert-Butyldimethylsilyl chloride(TBDMSCl), Dimethylformamide (DMF), imidazole, 92%; (ii) acetic anhydride, 4-Dimethylaminopyridine(DMAP), 3 h, 91%; (iii) $AcOH/H_2O$, THF, rt, 12 h, 91%; (iv) PCC, DCM, rt, 2h, 92%; (v) $NaClO_2/NaH_2PO_4$, 2-methylbut-2-ene, *t*-BuOH, rt, 2 h; 79% (vi) 1) Lawesson's reagent, DCM, microwave, 6 min; 2) NaOH, methanol, -78 ℃, 60% for the last two steps.

Synthesis of 2-(4-((tert-butyldimethylsilyl)oxy)-2-methylbutan-2-yl)phenol (**9**). To a 20-ml vial was added 2-(4-hydroxy-2-methylbutan-2-yl)phenol (**8**, 2.5 g, 14 mmol), imidazole (2.8 g, 42 mmol), TBDMSCl (4.2 g, 28 mmol) and DMF (7 ml). The mixture was stirred for 5 min at room temperature, then quenched with the addition of H_2O (100 mL), and extracted with ethyl acetate (2×150 mL). The combined organic phase was washed by saturated $NaHCO_3$ (2×150 mL) and dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to give the crude product, which was purified by recrystallization in methanol to give a white solid (3.8 g, 92%). ^{1}H NMR (400 MHz $CDCl_3$): δ 7.19 (d, *J* = 7.6 Hz, 1H), 7.08-7.04 (m, 1H), 6.86-6.83 (m, 1H), 6.66 (d, *J* = 7.6 Hz, 1H), 6.10 (s, 1H), 3.51 (t, *J* = 7.6 Hz, 2H), 2.17 (t, *J* = 7.6 Hz, 2H), 1.42 (s,

6H), 0.88 (s, 9H), 0.02 (s, 6H). ^{13}C NMR ($CDCl_3$) δ 154.9, 134.1, 127.7, 127.3, 120.2, 116.9, 61.7, 43.3, 36.7, 29.0, 26.1, 18.5, -5.1. HRMS calcd for $C_{17}H_{30}O_2Si$ $[M+H]^+$295.2088, found: 295.2075.

Synthesis of 2-(1-((tert-butyldimethylsilyl)oxy)-2-methylpropan-2-yl)phenyl acetate (**10**). To a solution of 2-(4-((tert-butyldimethylsilyl)oxy)-2-methylbutan-2-yl)phenol (**9**, 1.53 g, 5.6 mmol) in DCM (10 mL), was added acetic anhydride (1.63 g, 3 mmol), Et_3N (1.62 g, 16 mmol) and DMAP (0.29 g, 2.4 mmol). The mixture was stirred at room temperature for 2 h. The reaction was quenched with H_2O (10 mL) and extracted with ethyl acetate (2 × 50 mL). The combined organic phase was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to give the crude product, which was then purified by column chromatography (hexane: ethyl acetate =100:1) to obtain colorless oil (1.6 g, 91%). ^{1}H NMR ($CDCl_3$): δ 7.33 (dd, *J* = 7.6 Hz, 1.2 Hz, 1H), 7.25-7.21 (m, 1H), 7.18-7.14 (m, 1H), 7.00 (dd, *J* = 7.6 Hz, 1.2 Hz, 1H), 3.41 (t, *J* = 7.6 Hz, 2H), 2.33 (s, 3H) 2.01 (t, *J* = 7.6 Hz, 2H), 1.37 (s, 6H), 0.84 (s, 9H), -0.04 (s, 6H). ^{13}C NMR ($CDCl_3$) δ 169.5, 149.2, 139.0, 128.2, 127.2, 125.8, 124.1, 60.6, 44.5, 36.9, 29.3, 26.1, 21.8, 18.4, -5.2. HRMS cald for $C_{19}H_{32}O_3Si$ $[M+H]^+$337.2193, found: 337.2201.

Synthesis of 2-(4-hydroxy-2-methylbutan-2-yl)phenyl acetate (**11**). To a solution of 2-(1-((tert-butyldimethylsilyl)oxy)-2-methylpropan-2-yl)phenyl acetate (**10**) in 3 ml THF (1.50 g, 4.46 mmol), was added H_2O (3 mL) and AcOH (9 mL). The reaction mixture was stirred at room temperature for 4 h, quenched with H_2O (10 mL), and extracted with ethyl acetate (2×50 mL). The combined organic phase was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to give the crude product, which was purified by column chromatography (hexane: ethyl acetate =2:1) to obtain colorless oil (900 mg, 91%). ^{1}H NMR ($CDCl_3$): δ 7.32 (dd, *J* = 7.6 Hz, 1.6 Hz, 1H), 7.25-7.21 (m, 1H),7.18-7.14 (m, 1H), 6.98 (dd, *J* = 7.6 Hz, 1.6 Hz, 1H), 3.40 (t, *J* = 7.6 Hz, 2H), 2.34 (s, 3H), 1.99 (t, *J* = 7.6 Hz, 2H), 1.37 (s, 6H). ^{13}C NMR ($CDCl_3$) δ 169.9, 149.1, 138.8, 128.1, 127.3, 126.0, 124.2, 60.1, 44.4, 36.7, 29.2, 21.8. For $C_{13}H_{18}O_3$ $[M+H]^+$223.1329, found: 223.1333.

Synthesis of 2-(2-methyl-4-oxobutan-2-yl)phenyl acetate (**12**). To a solution of PCC (1.55 g, 7.2 mmol) in DCM (5 mL), a solution of (2-(4-hydroxy-2-methylbutan-2-yl)phenyl acetate (**11**, 710 mg, 3.20 mmol) in DCM (5 mL) was added dropwise at room temperature. After 2 h, the

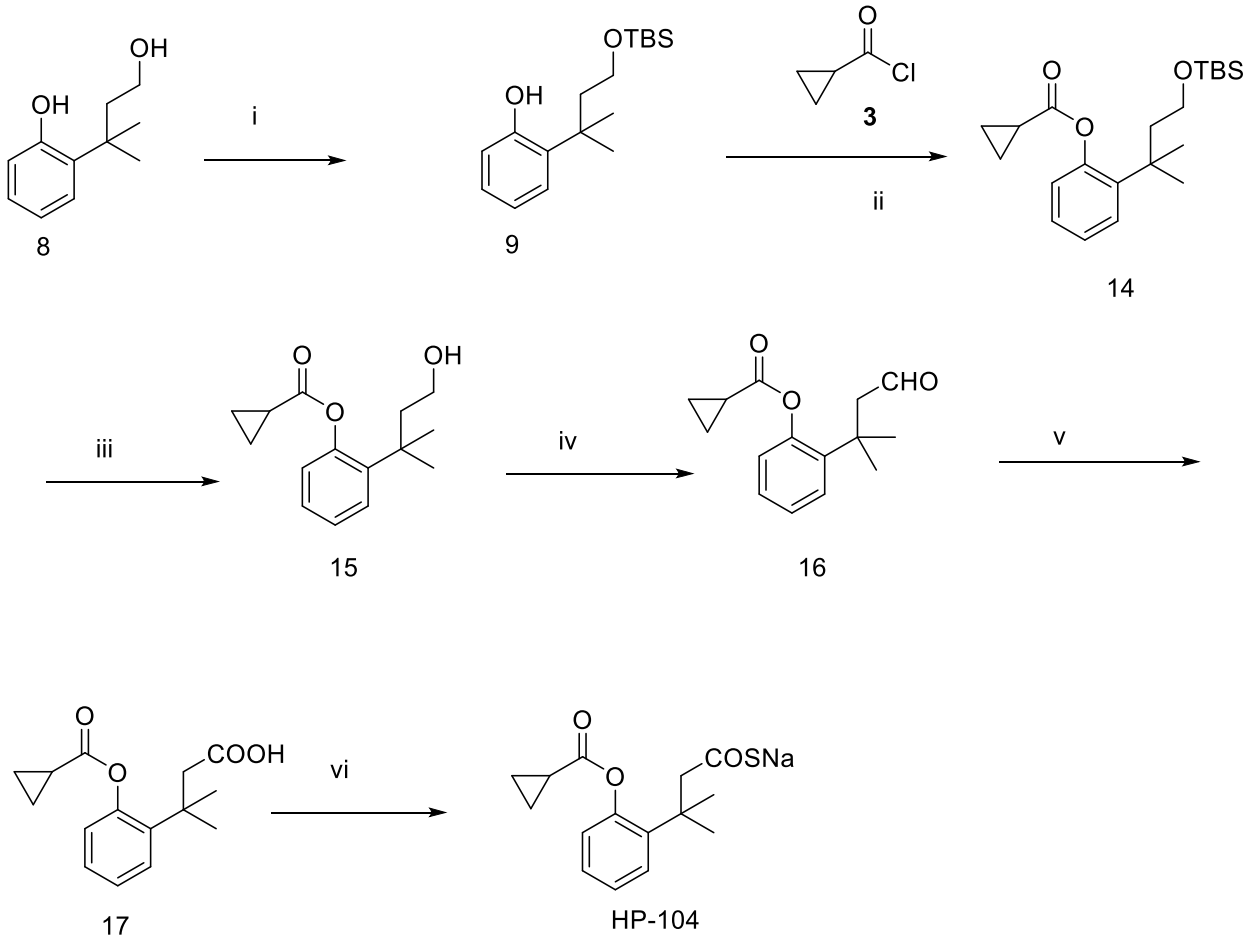
mixture was directly subjected to column chromatography (hexane: ethyl acetate =10:1) to obtain the pure product as colorless oil (650 mg, 92%). ^{1}H NMR ($CDCl_3$): δ 9.45 (t, *J* = 2.8 Hz, 1H), 7.38 (dd, *J* = 7.6 Hz, 1.6 Hz, 1H), 7.30-7.26 (m, 1H), 7.22-7.18 (m, 1H), 7.05 (dd, *J* = 7.6 Hz, 1.6 Hz, 1H), 2.79 (d, *J* = 2.8 Hz, 2H), 2.36 (s, 3H), 1.46 (s, 6H). ^{13}C NMR ($CDCl_3$): δ 202.8, 169.3, 149.0, 137.5, 128.0, 127.8, 126.2, 124.5, 54.6, 36.3, 29.1, 21.8. HRMS calcd for $C_{13}H_{16}O_3$ $[M+H]^+$221.1172, found: 221.1178.

Synthesis of 3-(2-acetoxyphenyl)butanoic acid (**13**). To a solution of 2-(2-methyl-4-oxobutan-2-yl)phenyl acetate (**12**, 600 mg, 2.73 mmol) in *t*-BuOH (12 mL) and 2-methylbut-2-ene (2.5 mL) was added dropwise $NaClO_2$ (564 mg, 6.27 mmol) in 0.67M NaH_2PO_4 (2.0 mL) at room temperature. After 2 h, the reaction was quenched with H_2O (20 mL), and extracted with ethyl acetate (2×50 ml). The combined organic phase was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to afford the crude product, which was purified by column chromatography (hexane: ethyl acetate =5:1) to obtain a white solid (510 mg, 79%). ^{1}H NMR ($CDCl_3$): δ 7.38 (dd, *J* = 8.0 Hz, 1.6 Hz, 1H), 7.27-7.23 (m, 1H), 7.19-7.15 (m, 1H), 7.03 (dd, *J* = 8.0 Hz, 1.6 Hz, 1H), 2.79 (s, 2H), 2.35 (s, 3H) 1.47 (s, 6H). ^{13}C NMR (CD_3OD) δ 175.2, 171.0, 150.4, 139.9, 128.9, 128.2, 126.7, 125.2, 46.6, 37.4, 29.1, 21.6. HRMS calcd for $C_{13}H_{16}O_4$ $[M+Na]^+$ 259.0941, found: 259.0945.

Synthesis of sodium 3-(2-acetoxyphenyl)-3-methylbutanethioate (**HP-103**). A solution of 3-(2-acetoxyphenyl)butanoic acid (**13**, 100 mg, 0.42 mmol) and Lawesson's reagent (85 mg, 0.21 mmol) in DCM (5 mL) in a sealed tube was subjected to microwave irradiation (100 ℃, 6 min). The mixture was directly subjected to column chromatography (hexane: ethyl acetate =20:1) to obtain the pure product as colorless oil, which was then dissolved in 5 ml methanol. To this solution 3 ml of 0.1 M NaOH methanol solution was added at -78 ℃. After 5 min, the mixture was allowed to warm to room temperature and the solvent was removed by vacuum. The final product was achieved by recrystallization in ether as a white solid (70 mg, 60%). ^{1}H NMR (CD_3OD): δ 7.39 (dd, *J* = 8.0Hz, 1.6 Hz, 1H), 7.26-7.22 (m, 1H), 7.19-7.15 (m, 1H), 7.04 (dd, *J* = 8.0Hz, 1.6 Hz, 1H), 3.18 (s, 2H), 2.36 (s, 3H) 1.44 (s, 6H). ^{13}C NMR ($CDCl_3$) δ 195.7, 169.3, 148.9, 137.5, 128.0, 127.8, 126.0, 124.2, 56.2, 37.5, 28.4, 21.8. HRMS calcd for $C_{13}H_{15}NaO_3S$ $[M-Na]^-$ 251.0747, found: 251.0742.

Synthesis of HP-104 (Scheme 4)

Scheme 4



Reagents and conditions (i) TBDMSCl , imidazole, DMF, rt, 92%; (ii) Et_3N, DCM, 0 ℃-rt, 12 h, 73%; (iii) AcOH/H_2O, THF, rt, 12 h, 85%; (iv) PCC, DCM, rt, 2 h 88%; (v) $NaClO_2$/NaH_2PO_4, 2-methylbut-2-ene, *t*-BuOH, rt, 2 h 65%; (vi) 1) Lawesson's reagent, DCM, microwave, 6 min; 2) NaOH, methanol, -78 ℃, 65% for the last two steps .

Synthesis of 2-(4-((tert-butyldimethylsilyl)oxy)-2-methylbutan-2-yl)phenol (**9**). To a 20-ml vial was added 2-(4-hydroxy-2-methylbutan-2-yl)phenol (**8**, 2.5 g, 14 mmol), imidazole (2.8 g, 42 mmol), TBDMSCl (4.2 g, 28 mmol) and DMF (7 ml). The mixture was stirred for 5 min at room temperature, then quenched with the addition of H_2O (100 mL), and extracted with ethyl acetate (2×150 mL). The combined organic phase was washed by saturated $NaHCO_3$ (2×150 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give the crude product, which

was purified by recrystallization in methanol to give a white solid (3.8 g, 92%). ^{1}H NMR ($CDCl_3$): δ 7.19 (d, *J* = 7.6 Hz, 1H), 7.08-7.04 (m, 1H), 6.86-6.83 (m, 1H), 6.66 (d, *J* = 7.6 Hz, 1H), 6.10 (s, 1H), 3.51 (t, *J* = 7.6 Hz, 2H), 2.17 (t, *J* = 7.6 Hz, 2H), 1.42 (s, 6H), 0.88 (s, 9H), 0.02 (s, 6H). ^{13}C NMR ($CDCl_3$) δ 154.9, 134.1, 127.7, 127.3, 120.2, 116.9, 61.7, 43.3, 36.7, 29.0, 26.1, 18.5, -5.1. HRMS calcd for $C_{17}H_{30}O_2Si$ $[M+H]^+$295.2088, found: 295.2075.

Synthesis of 2-(4-((tert-butyldimethylsilyl)oxy)-2-methylbutan-2-yl)phenyl cyclopropanecarboxylate (**14**). To a solution of 2-(4-((tert-butyldimethylsilyl)oxy)-2-methylbutan-2-yl)phenol (**9**, 2.0 g, 6.8 mmol) and Et_3N (1.4 g, 13.6 mmol) in DCM (150 mL) was added dropwise cyclopropanecarbonyl chloride (**3**, 1.46 g, 13.6 mmol) at 0 ℃. The mixture was allowed to warm to room temperature and stirred for 12 h. Then the reaction was quenched with the addition of H_2O (100 mL), and solution was extracted with ethyl acetate (2×150 mL). The combined organic phase was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to give the crude product, which was purified by column chromatography (hexane: ethyl acetate =50:1) to give a colorless oil (1.8 g, 73%). ^{1}H NMR ($CDCl_3$): δ 7.32 (dd, *J* = 7.6 Hz, 1.6 Hz, 1H), 7.23-7.19 (m, 1H), 7.16-7.12 (m, 1H), 6.98 (dd, *J* = 7.6 Hz, 1.6 Hz, 1H), 3.41 (t, *J* = 7.6 Hz, 2H), 2.03 (t, *J* = 7.6 Hz, 2H), 1.92-1.85 (m, 1H), 1.38 (s, 6H), 1.19-1.18 (m, 2H), 1.05-1.00 (m, 2H), 0.84 (s, 9H), -0.04 (s, 6H). ^{13}C NMR ($CDCl_3$) δ 173.5, 149.4, 139.1, 128.1, 127.1, 125.6, 124.2, 60.7, 44.5, 36.9, 29.2, 26.1, 18.3, 13.5, 9.1, -5.2. HRMS calcd for $C_{21}H_{34}O_3Si$ $[M+H]^+$363.2350, found: 363.2348.

Synthesis of 2-(4-hydroxy-2-methylbutan-2-yl)phenyl cyclopropanecarboxylate (**15**). To a solution of 2-(4-((tert-butyldimethylsilyl)oxy)-2-methylbutan-2-yl)phenyl cyclopropanecarboxylate (**14**, 1.7 g, 4.69 mmol） in THF (20 mL) was added H_2O (20 mL) and AcOH (60 mL). The reaction mixture was stirred at room temperature for 4 h, quenched with H_2O (50 mL), and extracted with ethyl acetate (2 × 150 mL). The combined organic phase was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure, and purified silica gel column chromatography (hexane: ethyl acetate =10:1) as colorless oil (1.1 g, 95%). ^{1}H NMR (400 MHz $CDCl_3$): δ 7.32 (dd, *J* = 7.6 Hz, 1.6 Hz, 1H), 7.24-7.20 (m, 1H), 7.18-7.13 (m, 1H), 6.96 (dd, *J* = 7.6 Hz, 1.6 Hz, 1H), 3.42 (t, *J* = 7.6 Hz, 2H), 2.03 (t, *J* = 7.6 Hz, 2H), 1.93-1.87 (m, 1H), 1.39 (s, 6H), 1.21-1.17 (m, 2H), 1.07-1.02 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 174.1, 149.4, 139.0,

128.1, 127.3, 125.9, 124.3, 60.3, 44.4, 36.9, 29.2, 13.6, 9.2. For $C_{15}H_{20}O_3$ $[M+H]^+$249.1485, found: 249.1485.

Synthesis of2-(2-methyl-4-oxobutan-2-yl)phenyl cyclopropanecarboxylate (**16**). To a solution of PCC (2.2 g, 10.0 mmol) in DCM (20 mL) was added dropwise 2-(4-hydroxy-2-methylbutan-2-yl)phenyl cyclopropanecarboxylate (**15**, 1.1 g, 4.4 mmol) in DCM (25 mL) at room temperature. After 2 h, the mixture was directly subjected to column chromatography (hexane: ethyl acetate =20:1) to obtain the pure product as colorless oil (0.95 g, 88%). ^{1}H NMR ($CDCl_3$): δ 9.45 (t, *J* = 2.8 Hz, 1H), 7.38 (dd, *J* = 8.0 Hz, 1.6 Hz, 1H), 7.29-7.25 (m, 1H), 7.21-7.17 (m, 1H), 7.03 (dd, *J* = 8.0 Hz, 1.6 Hz, 1H), 2.81 (d, *J* = 2.8 Hz, 2H), 1.93-1.86 (m, 1H), 1.47 (s, 6H), 1.21-1.17 (m, 2H), 1.08-1.04 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 202.9, 173.4, 149.2, 137.6, 127.9, 127.7, 126.0, 124.5, 54.5, 36.3, 29.1, 13.5, 9.2. HRMS calcd for $C_{15}H_{18}O_3$ $[M+Na]^+$269.1148, found: 269.1149.

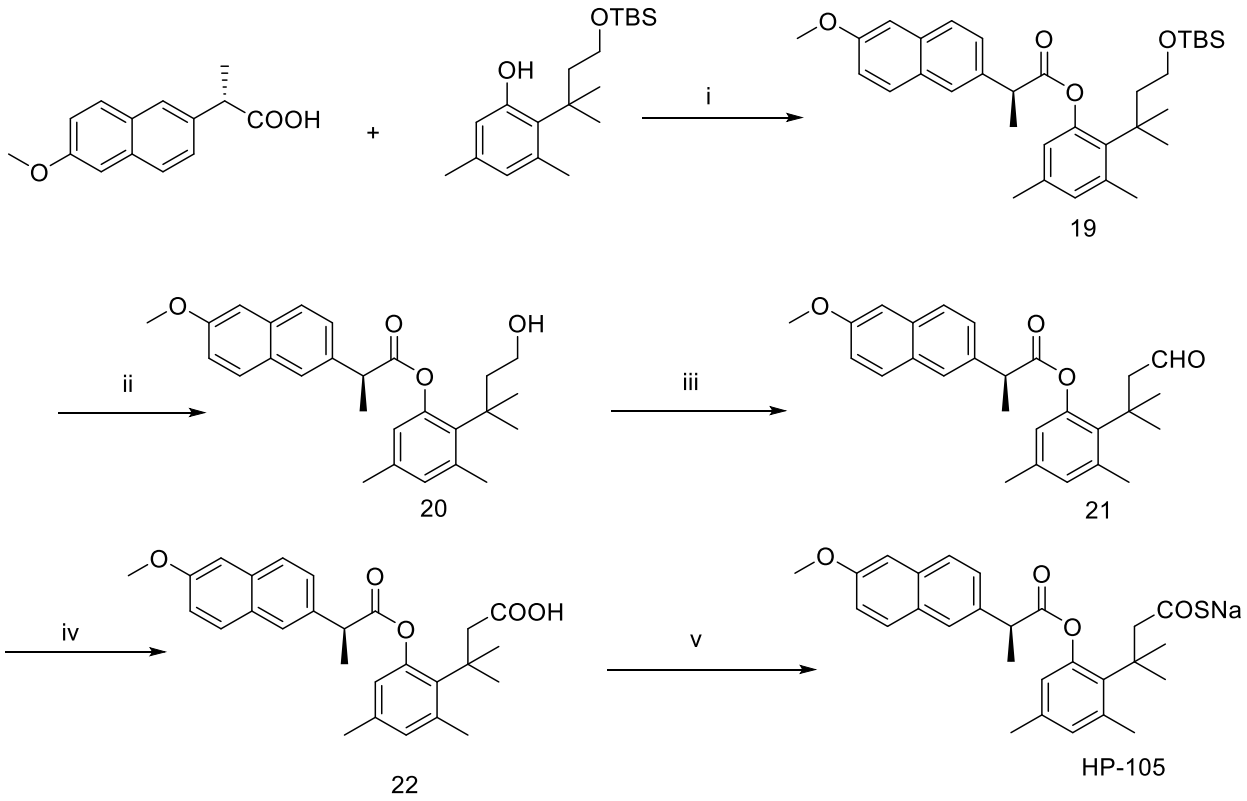
Synthesis of 3-(2-((cyclopropanecarbonyl)oxy)phenyl)-3-methylbutanoic acid (**17**). To a solution of 2-(2-methyl-4-oxobutan-2-yl)phenyl cyclopropanecarboxylate (**16**, 900 mg, 3.6 mmol) in *t*-BuOH (20 mL) and 2-methylbut-2-ene (4 mL) $NaClO_2$ (496 mg, 5.4 mmol) in 0.67 M NaH_2PO_4 (4 mL) was added dropwise at room temperature. After 2 h, the reaction mixture was quenched with H_2O (20 mL), and extracted with ethyl acetate (2 × 100 ml). The combined organic phase was dried over anhydrous Na_2SO_4 and then evaporated under reduced pressure to give the crude product, which was purified by column chromatography (hexane: ethyl acetate =10:1) to yield a white solid (610 mg, 65%). ^{1}H NMR (MeOH): δ 7.42 (dd, *J* = 7.6 Hz, 1.6 Hz, 1H), 7.23-7.14 (m, 2H), 6.97 (dd, *J* = 7.6 Hz, 1.6 Hz, 1H), 2.80 (s, 2H), 1.99-1.93 (m, 1H), 1.47 (s, 6H), 1.13-1.07 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 177.6, 173.6, 149.1, 138.2, 127.8, 127.5, 125.8, 124.1, 45.8, 36.7, 28.4, 13.5, 9.2. HRMS calcd for $C_{15}H_{18}O_4$ $[M+H]^+$263.1278, found: 263.1279.

Synthesis of sodium 3-(2-((cyclopropanecarbonyl)oxy)phenyl)-3-methylbutanethioate (**HP-104**). To a solution of 3-(2-((cyclopropanecarbonyl)oxy)phenyl)-3-methylbutanoic acid (**17**, 120 mg, 0.46 mmol) in DCM (5 mL) was added Lawesson's reagent (92 mg, 0.23 mmol). The mixture was heated in a microwave at 100℃ for 6 min. The mixture was directly subjected to column chromatography (hexane: ethyl acetate =20:1) to obtain the pure product as colorless oil, which was then dissolved in 5 ml methanol. Then 2.5 ml of 0.1 M NaOH methanol solution was added to the reaction solution at -78 ℃. After 5 min, the mixture was allowed to warm to room temperature and the solvent was removed by vacuum. The final product was achieved by

recrystallization in ether as a white solid (90 mg, 65%). ^{1}H NMR ($CDCl_3$): δ 7.38 (d, *J* = 7.2 Hz, 1H), 7.28-7.17 (m, 2H), 7.04 (d, *J* = 8.0 Hz, 1H), 4.41 (s, 1H), 3.11 (s, 2H), 1.97-1.90 (m, 1H), 1.48 (s, 6H), 1.22-1.20 (m, 2H), 1.09-1.06 (m, 2H); ^{13}C NMR ($CDCl_3$): 195.8, 173.4, 149.1, 137.6, 128.0, 127.7, 125.9, 124.2, 56.3, 37.5, 28.4, 13.5, 9.3. HRMS calcd for $C_{15}H_{17}NaO_3S$ $[M+H]^+$301.0869, found: 301.0871.

Synthesis of HP-105 (Scheme 5)

Scheme 5



Reagents and conditions (i) EDC/DMAP, DCM, rt, 2 h, 88%; (ii) AcOH/H_2O, THF, rt, 12 h, 87%; (iii) PCC, DCM, rt, 2 h, 81%; (iv) $NaClO_2$/NaH_2PO_4, 2-methylbut-2-ene, *t*-BuOH, rt, 2 h, 66%; (v) 1) Lawesson's reagent, DCM, microwave, 6 min; 2) NaOH, methanol, -78 ℃, 65% for the last two steps

Synthesis of 2-(4-((tert-butyldimethylsilyl)oxy)-2-methylbutan-2-yl)-3,5-dimethylphenyl (S)-2-(6-methoxynaphthalen-2-yl)propanoate (**19**). To a solution of 2-(4-((*tert*-butyldimethylsilyl)oxy)-2-methylbutan-2-yl)-3,5-dimethylphenol (**2**, 1.27 g, 3.9 mmol), (S)-2-(6-methoxynaphthalen-2-yl)propanoic acid (**18**, 1.00 g, 4.4 mmol) and DMAP (100 mg, 0.8 mmol) in DCM (50 mL) was added EDC (1.62 g, 8.7 mmol). The mixture was stirred at room temperature for 2 h, then quenched with the addition of H_2O (50 mL), and extracted with DCM (2×50 mL). The combined organic phase was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to give the crude product, which was purified by column chromatography (hexane: ethyl acetate =50:1) to give a colorless oil (1.86 g, 88%). ^{1}H NMR ($CDCl_3$): δ 7.77-7.72 (m, 3H), 7.50 (dd, *J* = 8.4 Hz, *J* = 1.6 Hz, 1H), 7.17-7.14 (m, 2H), 6.74 (d, *J* = 1.2 Hz, 1H), 6.32 (d, J = 1.2 Hz, 1H), 4.04 (q, , *J* = 7.2 Hz, 1H), 3.93 (s, 3H), 3.40 (t, *J* = 7.2 Hz, 2H), 2.48 (s, 3H), 2.14 (s, 3H), 1.91 (t, *J* = 7.2 Hz, 2H), 1.69 (d, *J* = 7.2 Hz, 3H), 1.36 (d, *J* = 7.2 Hz, 6H), 0.84 (s, 9H), -0.06 (s, 6H); ^{13}C NMR ($CDCl_3$): 173.7, 157.8, 150.2, 138.3, 136.0, 134.9, 134.2, 134.0, 132.3, 129.5, 129.1, 127.4, 126.6, 126.5, 122.7, 119.2, 105.7, 60.9, 55.4, 46.3, 45.9, 39.2, 31.9, 31.9, 26.1, 25.4, 20.3, 18.6, 18.3, -5.2. HRMS calcd for $C_{33}H_{46}O_4Si$ $[M+H]^+$535.3238, found: 535.3239.

Synthesis of 2-(4-hydroxy-2-methylbutan-2-yl)-3,5-dimethylphenyl (S)-2-(6-methoxynaphthalen-2-yl)propanoate (**20**). To a solution of (2-(4-((tert-butyldimethylsilyl)oxy)-2-methylbutan-2-yl)-3,5-dimethylphenyl (S)-2-(6-methoxynaphthalen-2-yl)propanoate (**19**, 1.86 g, 3.5 mmol)in THF (15 mL) was added H_2O (20 mL) and AcOH (45 mL). The reaction mixture was stirred at room temperature for 12 h, quenched with H_2O (50 mL), and extracted with ethyl acetate (2 × 100 mL). The combined organic phase was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. Silica gel column chromatography (hexane: ethyl acetate =2:1) gave the product as colorless oil (1.27 g, 87%). ^{1}H NMR ($CDCl_3$): δ 7.79-7.74 (m, 3H), 7.52 (dd, *J* = 8.4 Hz, *J* = 1.6 Hz, 1H), 7.19-7.15 (m, 2H), 6.77 (d, *J* = 1.2 Hz, 1H), 6.35 (d, *J* = 1.2 Hz, 1H), 4.08 (q, *J* = 7.2 Hz, 1H), 3.93 (s, 3H), 3.44 (t, *J* = 7.2 Hz, 2H), 2.49 (s, 3H), 2.16 (s, 3H), 1.92-1.80 (m, 2H), 1.72 (d, *J* = 7.2 Hz, 3H), 1.37 (s, 6H); 13C NMR (CDCl3): 174.2, 157.9, 150.2, 138.4, 136.3, 134.6, 134.0, 134.0, 132.5, 129.4, 129.1, 127.5, 126.7, 126.5, 122.8, 119.3, 105.7, 60.6, 55.4, 46.3, 45.6, 39.2, 32.0, 25.5, 20.2, 18.5. HRMS calcd for $C_{27}H_{32}O_4$ $[M+Na]^+$443.2193, found: 443.2192.

Synthesis of 3,5-dimethyl-2-(2-methyl-4-oxobutan-2-yl)phenyl (S)-2-(6-methoxynaphthalen-2-yl)propanoate (**21**). To a solution of PCC (1.2 g, 5.6 mmol) in DCM (15 mL) was added dropwise the solution of 2-(4-hydroxy-2-methylbutan-2-yl)-3,5-dimethylphenyl (S)-2-(6-methoxynaphthalen-2-yl)propanoate (**20**, 1.2 g, 2.8 mmol) in DCM (20 mL) at room temperature. After 2 h, the mixture was directly subjected to column chromatography (hexane: ethyl acetate =5:1) to obtain the pure product as colorless oil (0.97 g, 81%). ^{1}H NMR ($CDCl_3$): δ 9.36 (d, *J* = 2.4 Hz, 1H), 7.77-7.73 (m, 3H), 7.49 (dd, *J* = 8.8 Hz, *J* = 1.6 Hz, 1H), 7.19-7.14 (m, 2H), 6.79 (s, 1H), 6.43 (s, 1H), 4.06 (q, *J* = 7.2 Hz, 1H), 3.93 (s, 3H), 2.54 (t, *J* = 2.4 Hz, 2H), 2.48 (s, 3H), 2.18 (s, 3H), 1.71 (d, *J* = 7.2 Hz, 3H), 1.40 (s, 3H), 1.35 (s, 3H); 13C NMR (CDCl3): 203.22, 173.5, 157.9, 149.9, 137.8, 136.9, 134.3, 134.0, 132.9, 132.7,129.4, 129.1, 127.6, 126.7, 126.4, 123.0, 119.4, 105.7, 56.4, 55.4, 46.3, 38.2, 31.6, 31.4, 25.6, 20.3, 18.4. HRMS calcd for $C_{27}H_{30}O_4$ $[M+Na]^+$441.2036, found: 441.2033.

Synthesis of (S)-3-(2-((2-(6-methoxynaphthalen-2-yl)propanoyl)oxy)-4,6-dimethylphenyl)-3-methylbutanoic acid (**22**). To a solution of 3,5-dimethyl-2-(2-methyl-4-oxobutan-2-yl)phenyl (S)-2-(6-methoxynaphthalen-2-yl)propanoate (**21**, 0.96 g, 2.3 mmol) in *t*-BuOH (18 mL) and 2-methylbut-2-ene (3 mL) was added dropwise $NaClO_2$ (330 mg, 3.4 mmol) in 0.67 M NaH_2PO_4 (3.6 mL) at room temperature. After 2 h, the reaction mixture was quenched with H_2O (20 mL), and extracted with ethyl acetate (2 × 50 ml). The combined organic phase was dried over anhydrous Na_2SO_4 and then evaporated under reduced pressure to give the crude product, which was purified by column chromatography (hexane: ethyl acetate =2:1) to yield a white solid (650 mg, 66%). ^{1}H NMR ($CDCl_3$): δ7.77-7.72 (m, 3H), 7.50 (dd, *J* = 8.8 Hz, *J* = 2.0 Hz, 1H), 7.16-7.13 (m, 2H), 6.75 (s, 1H), 6.40 (s, 1H), 4.09 (q, , *J* = 7.2 Hz, 1H), 3.92 (s, 3H), 2.68-2.55 (m, 2H), 2.49 (s, 3H), 2.15 (s, 3H), 1.70 (d, *J* = 7.2 Hz, 3H), 1.44 (s, 3H), 1.38 (s, 3H); 13C NMR (CDCl3): 176.8, 173.7, 157.9, 150.0, 138.1, 136.4, 134.5, 134.0, 133.5, 132.5,129.5, 129.1, 127.5, 126.7, 126.5, 122.7, 119.2, 105.7, 55.4, 47.2, 46.3, 38.8, 31.4, 31.2, 25.5, 20.3, 18.4. HRMS calcd for $C_{27}H_{30}O_5$ $[M+Na]^+$457.1985, found: 457.1983.

Synthesis of Sodium (S)-3-(2-((2-(6-methoxynaphthalen-2-yl)propanoyl)oxy)-4,6-dimethylphenyl)-3-methylbutanethioate (**HP-105**). To a solution of (S)-3-(2-((2-(6-methoxynaphthalen-2-yl)propanoyl)oxy)-4,6-dimethylphenyl)-3-methylbutanoic acid (**22**, 180

mg, 0.41 mmol) in DCM (5 mL) was added Lawesson's reagent (83 mg). The mixture was heated in a microwave at 100 ℃ for 6 min. The mixture was directly subjected to column chromatography (hexane: ethyl acetate =15:1) to obtain the pure product as colorless oil, which was then dissolved in 5 ml methanol. Then 2.6 ml 0.1 M NaOH methanol solution was added at -78 ℃. After 5 min, the mixture was allowed to warm to room temperature and the solvent was removed by vacuum. The final product was achieved by recrystallization in ether as white solid (140 mg, 72%) ^{1}H NMR ($CDCl_3$): δ 7.81-7.74 (m, 3H), 7.52 (dd, *J* = 8.8 Hz, *J* = 2.0 Hz, 1H), 7.20-7.15 (m, 2H), 6.76 (d, *J* = 1.6 Hz 1H), 6.49 (d, *J* = 1.6 Hz 1H), 4.14 (q, *J* = 7.2 Hz, 1H), 3.94 (s, 3H), 2.80-2.64 (m, 2H), 2.48 (s, 3H), 2.18 (s, 3H), 1.74 (d, *J* = 7.2 Hz, 3H), 1.38 (s, 3H), 1.30 (s, 3H); 13C NMR (CDCl3): 196.2, 173.4, 158.0, 150.1, 138.1, 136.5, 134.4, 134.1, 132.8, 132.6, 129.5, 129.1, 17.7, 126.8, 126.5, 122.8, 119.4, 105.8, 58.1, 55.4, 46.4, 39.7, 31.5, 31.3, 25.6, 20.4, 18.3. HRMS calcd for $C_{27}H_{29}O_4SNa$ $[M+H]^+$: 473.1757, found: 473.1756.

H_2S release from HPs

Stock solution preparation. HPs stock solution: HPs were dissolved in DMSO to afford 20 mM HPs stock solutions. Esterase stock Solution: 6.0 mg Esterase (18 unit/mg esterase from porcine liver, PLE, Aldrich, E3019) was dissolved in 1.080 ml PBS to provide a 100 unit/mL esterase stock solution. WSP-5 stock solution: WSP-5 was dissolved in DMSO to prepare a 2.5 mM stock solution. CTAB was dissolved in ethanol to prepare a 100 mM stock solution.

H_2S concentration measurement by an electrode probe ISO-H2S-2.

HPs (Final Conc. 200 μM) was added to an incubation chamber (World Precision Instruments; WPI) containing phosphate buffer (10 mM; pH 7.4, 10 mL), and esterase (1 unit/ mL) at 37 ℃. H_2S formation was detected with the use of a 2-mm H_2S-selective microelectrode (ISO-H2S-2; WPI) attached to an Apollo 1100 Free Radical Analyser (WPI) and shown as picoamps current generated. A standard curve (using Na_2S $9H_2O$) was generated by following literature procedures,[4] but using PBS containing esterase at 37 ℃ .

H_2S measurement by a fluorescent probe WSP-5.

HP-101 (final concentration: 200 μM) or other control compounds were added to PBS (10 mL) buffer containing esterase (1 unit/ mL) at 37 ℃. After 15 minutes, aliquots of 100 μL samples were taken out and added into 100 μL PBS containing 50 uM WSP-5 and 100 μM CTAB in 96-well plate. After mixing and standing for 5 min at room temperature, the fluorescent intensities at 535 nm were recorded by a plate reader with excitation at 485 nm.

H_2S release from HP-101 in cell culture media

HP-101 (200 μM) was added to an incubation chamber (World Precision Instruments; WPI) containing cell culture media (10mL) at 37 ℃. H_2S formation was detected with the use of a ISO-H_2S-2 attached to an Apollo 1100 Free Radical Analyser and shown as picoamps current generated (Figure 1). A standard curve (using Na_2S $9H_2O$) was also generated under the same conditions.[4] The results (Figure 1) indicated that HP-101 in the DMEM did not release H_2S; however, when HP-101 was added into the media collected after overnight of cell culturing, H_2S was released at a moderate rate, presumably due to the presence of esterases produced by the cells.

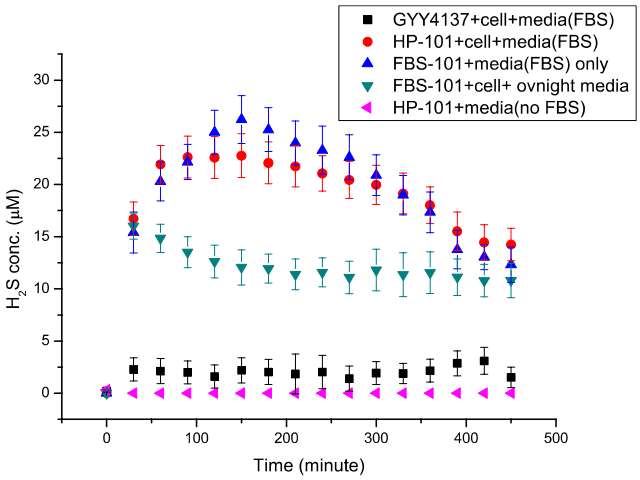


Figure 1. Standard curves for H_2S release from HP-101 (200 μM) in cell culture media with or without FBS as detected using an electrode probe. (n= 3, p= 0.95)

H_2S release from HP-105 measurement

To 9.9 mL phosphate buffer (pH 7.4) solution was added 11.1 mg (200 unit) PLE, followed by the addition of 100 μL 20 mM HP-105 stock solution (Final Conc.: 200μM). The resultant

solution was sealed and stirred at 37 ℃. At every 30 min, 200 μL of reaction solution was taken into a 1.5 mL vial containing 200 μL zinc acetate (1 %, w/v). Then the vial was centrifuged for 10 min. (14.5 × 1000 rp). Removed the supernatant and washed the precipitation with PBS solution (100 μL × 2). Then 600μL N,N-dimethyl-1,4-phenylenediaminesulfate (0.2% w/v in 20% H_2SO_4 solution) and 50 μL ferric chloride (10% w/v in 0.2% H_2SO_4 solution) was added to the vial. Then the vial was centrifuged for 5 min (14.5 × 1000 rp). The absorbance (at 740 nm) of the resulting solution was measured (after stirring for 10 min). H2S concentration was calculated based on a calibration curve of NaHS.

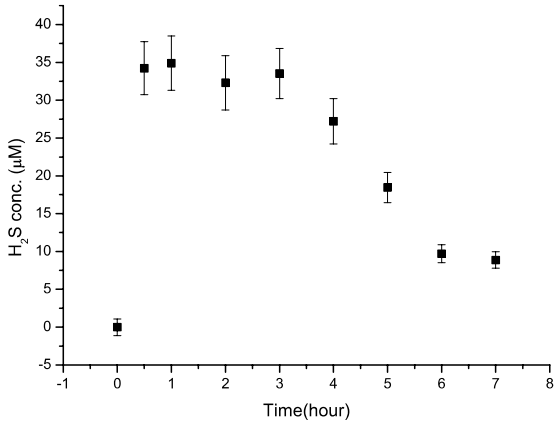


Figure 2. Standard curves for H_2S release from HP-105 (200 μM) in PBS at 37 ℃ with 20 unit/ mL PLE. (n= 3, p= 0.95)

Kinetic studies of esterase trigger lactone formation

Esterase triggered lactone formation from HP-101 as monitored by LC-MS/MS (Figure 3)

HP-101 (final Conc. 200 μM) was added to PBS (10 mL) with 1 unit/mL esterase at 37 ℃. Reaction mixture (10 μL) was taken out every 3 minutes and added into a vial containing 990 uL methanol at -78 ℃ for 5 minutes. The mixture (14.5 ×1000 rp) was centrifuged, and the supernatant was used as the sample for LC-MS/MS analysis (Agilent 1100 LC, 6410 TripleQ MS/MS, Ion transition: 205.0/135.0, positive mode).

All LC-MS/MS samples were analyzed using liquid chromatography tandem mass spectrometric method (Agilent 6410 series). Auto-sampler temperature was set at 10 ℃, a positive ionization mode with multiple reaction monitoring (MRM, *m/z* Q1/Q3) of lactone (*m/z* 205.0/135.0, RT

1.4min) was employed. The ion spray voltage was set at 3500 V, ionization temperature set as 300 ℃ and drying gas flow rate at 10 L/min. Data acquisition and quantitation were performed using Mass Hunter software (Agilent Technologies). Separation was achieved using HP1100 series LC (Agilent Technologies, Wilmington, DE) equipped with a photodiode array (PDA) detector, using an Agilent Zorbax reversed-phase (SB-C18, 3.0×250 mm, 5.0 μm) column. A gradient method was employed to separate the individual GE components using mobile phase A (0.1% formic acid in water) and mobile phase B (ACN). The gradient elution method with 30% B at 0 min, 90% B at 20 min, held for 10 min, back to 30% B at 40 min with a flow rate of 0.4 mL/min. An injection volume of 10 μL was used for analysis.

The results (Figure 3) showed that about 190 μM of the lactone product was formed after treatment with esterase for 25 min. Such results indicate that about 95% of H_2S was released in that period.

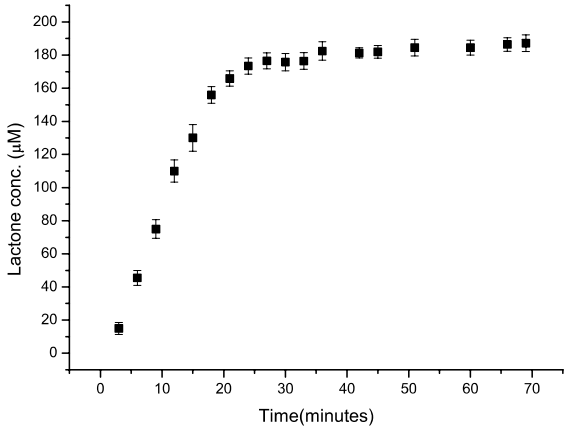


Figure 3. Esterase-triggered lactone formation from HP-101 as monitored with LC-MS/MS. (n= 3, p= 0.95)

Esterase triggering lactone formation from HPs as monitored with HPLC

HPs (final Conc. 200 μM) was added to PBS (10 mL) with 1 unit/mL esterase at 37 ℃. 200 μL reaction mixture was taken out every 10 minutes and added into a vial containing 600 μL ethanol at -78 ℃ for 5 minutes. The mixture (14.5 ×1000 rp, 90 seconds) was centrifuged, and the supernatant was used as the sample for HPLC. 200 μL HPLC samples were injected into Shimadzu Prominence UFLC (column: Waters C18 3.5 μM, 4.6×100 mm, injection loop volume:

20μL). The mobile phase was acetonitrile (ACN)/H_2O (pH=4.0) with ratios defined in the table below. (Table 1)

	HP-101	HP-102	HP-103	HP-104	HP-105
Eluent conditions	50% ACN 0~20 min	60% ACN 0~20 min	45% ACN 0~20 min	55% ACN 0~20min	Method b
Retention time of HPs (min)	13.6 ±0.2	9.7 ±0.2	7.7 ±0.2	8.3 ±0.2	20.7 ±0.3
Retention time of the lactones (min)	10.7 ±0.2	5.5 ±0.2	8.5 ±0.2	6.1 ±0.2	9.6 ±0.3

Method b: 45% ACN, 0~10min; 45%~75% ACN, 10~15 min; 75% CAN, 15-20min; 75%~45% CAN, 20~25min.

Table 1. HPLC monitored esterase triggering lactone formation of HPs. (n= 3, p= 0.95)

Stability Studies of the thioacid group

To a solution of 5 mL deuterated PBS (1X pH=7.4), 50.0 mg of potassium thioacetate (0.438 mmol) and 4.0 mg of acetic acid (0.0658 mmol) were added. The mixture was incubated at 37 ℃ for 48 hours. The mixture was analyzed by ^1H NMR at 0- and 48-hour time points, respectively. The spectrums were shown below (Figure 4). At 0 h, the integration ratio between the methyl protons in thioacetate (CH_3: 2.48 ppm) and that of acetic acid (CH_3: 1.94 ppm) is 6.7, which is consistent of the ratio of the compounds we added, and after 48 hours, the ratio did not change. Such results indicate that the thioacid group is stable under the conditions of the study.

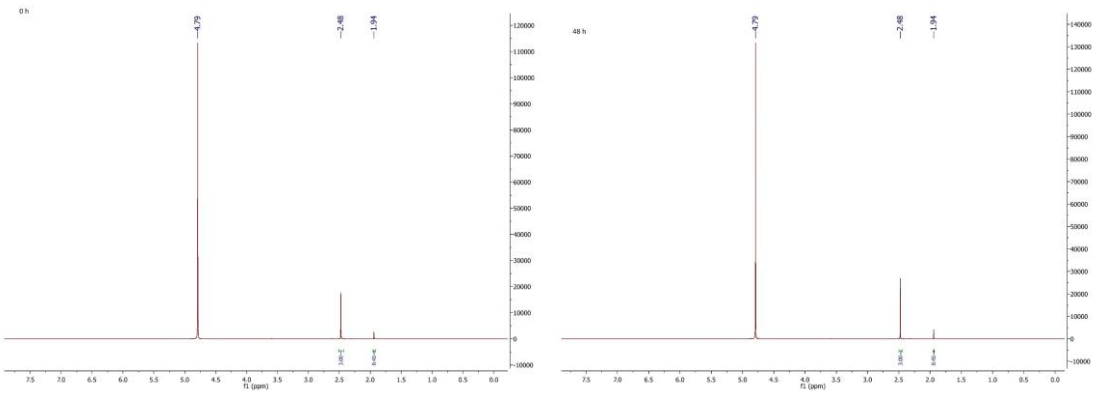


Figure 4. ^{1}H NMR spectra of reaction mixture at the 0- and 48-hour time points.

Cell culture

RAW 264.7 (ATCC® TIB-71™) mouse macrophage cells were used in the studies. The RAW 264.7 cells were maintained in DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 10% fetal bovine serum (MidSci; S01520HI) and 1% penicillin-streptomycin (Sigma-Aldrich; P4333) at 37 ℃ with 5% CO_2.

Cytotoxicity study

The RAW 264.7 cells were seeded in 96-well plate one day before the experiment. Different concentrations of HP or iHP compounds was directly dissolved in cell culture media and added into the RAW 264.7 cell culture. The cells were then incubated with the compound for 24 hours at 37 ℃ with 5% CO_2. The cell viability was tested by the MTT assay. Specifically, after 24 hr of incubation, 0.5 mg/mL MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide) was added into the cell culture for 4 hours. Thereafter, the supernatant was removed and 100 μL DMSO was added into the wells containing the cells. After shaking gently for 3 minutes, absorbance at 570 nm was read by a plate reader (Figure 5).

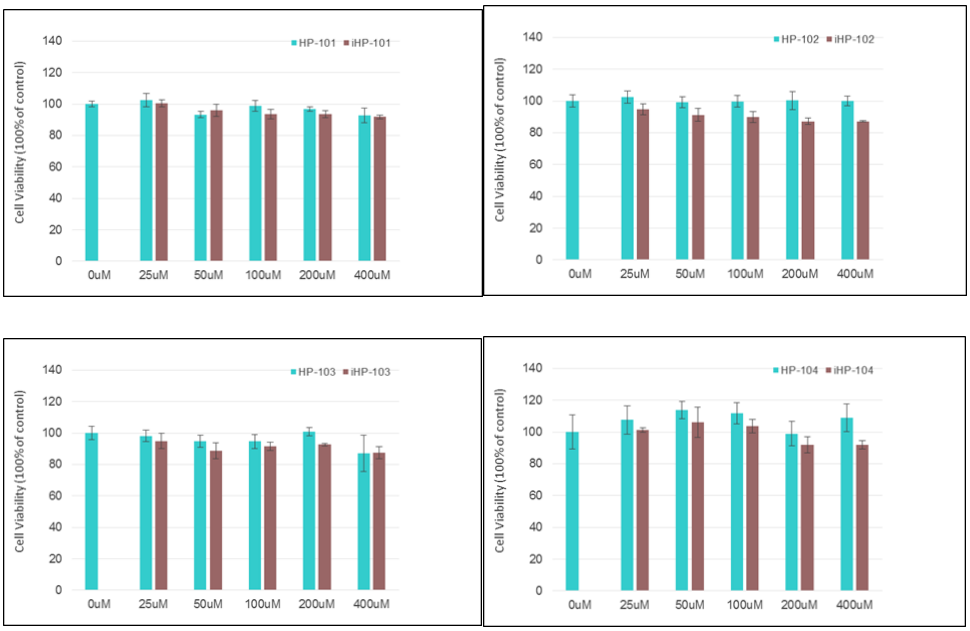


Figure 5. Cytotoxicity of HPs (n= 4, p=0.95)

Anti-inflammation study

The RAW 264.7 cells were seeded in the 48-well plate one day before the experiment. Lipopolysaccharide was added into the cell culture to initiate the inflammatory response in RAW 264.7 cells and to trigger the expression of cytokines. RAW 264.7 cells were co-treat with HPs or iHPs, 1unit/mL esterase and 1 μg/mL of LPS for 1 hour. Thereafter, the cell culture supernatant was collected. The concentrations of TNF-α in the cell culture supernatant was quantified by a commercial ELISA kit (ELISA Ready-SET-Go!® -eBioscience).

Reference

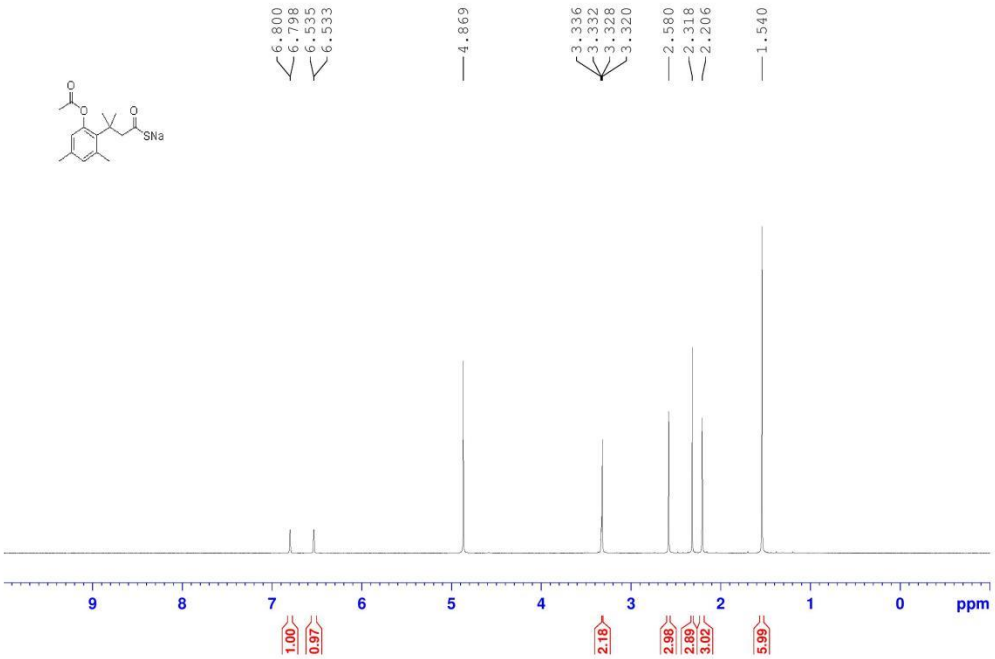
[1] a) K. L. Amsberry, A. E. Gerstenberger, R. T. Borchardt, *Pharm. Res.* **1991**, *8*, 455-461; b) K. L. Amsberry, R. T. Borchardt, *J. Org. Chem.* **1990**, *55*, 5867-5877.

[2] B. Peng, W. Chen, C. Liu, E. W. Rosser, A. Pacheco, Y. Zhao, H. C. Aguilar, M. Xian, *Chemistry* **2014**, *20*, 1010-1016.

[3] L. Li, M. Whiteman, Y. Y. Guan, K. L. Neo, Y. Cheng, S. W. Lee, Y. Zhao, R. Baskar, C. H. Tan, P. K. Moore, *Circulation* **2008**, *117*, 2351-2360.

[4] Y. Zhao, H. Wang, M. Xian, *J. Am. Chem. Soc.* **2011**, *133*, 15-17.

^{1}H and ^{13}C Spectra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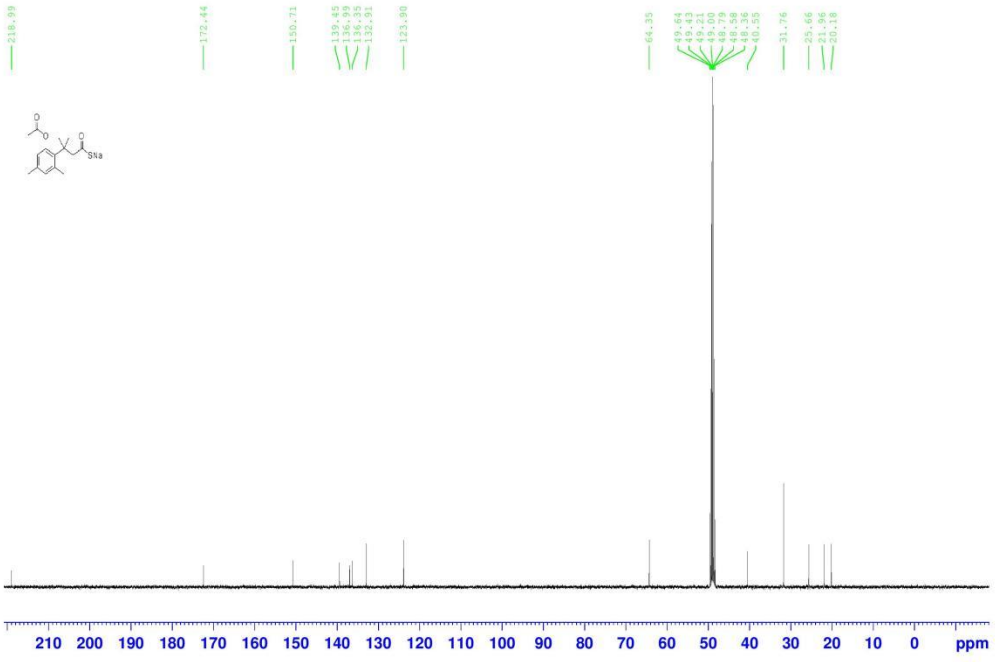
6.535
6.533
4.869
3.336
3.332
3.328
3.320
2.580
2.318
2.206
1.540
SNa
9
8
7
6
5
4
3
2
1
0
ppm
1.00
0.97
2.18
2.98
2.89
3.02
5.99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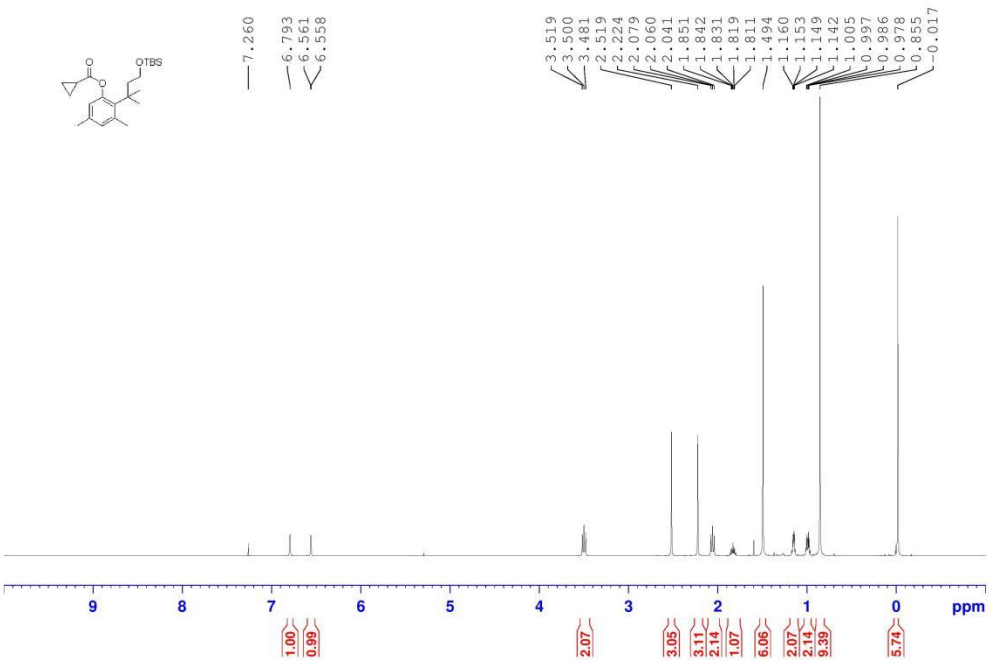
172.44
150.71
139.45
136.99
136.35
132.91
123.90
64.35
49.64
49.43
49.21
49.00
48.79
48.58
48.36
40.55
31.76
29.66
21.36
20.18
SNa
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm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6.561
6.558
3.519
3.500
3.481
2.519
2.224
2.079
2.060
2.041
1.851
1.842
1.831
1.819
1.811
1.494
1.160
1.153
1.149
1.142
1.005
0.997
0.986
0.978
0.855
0.017
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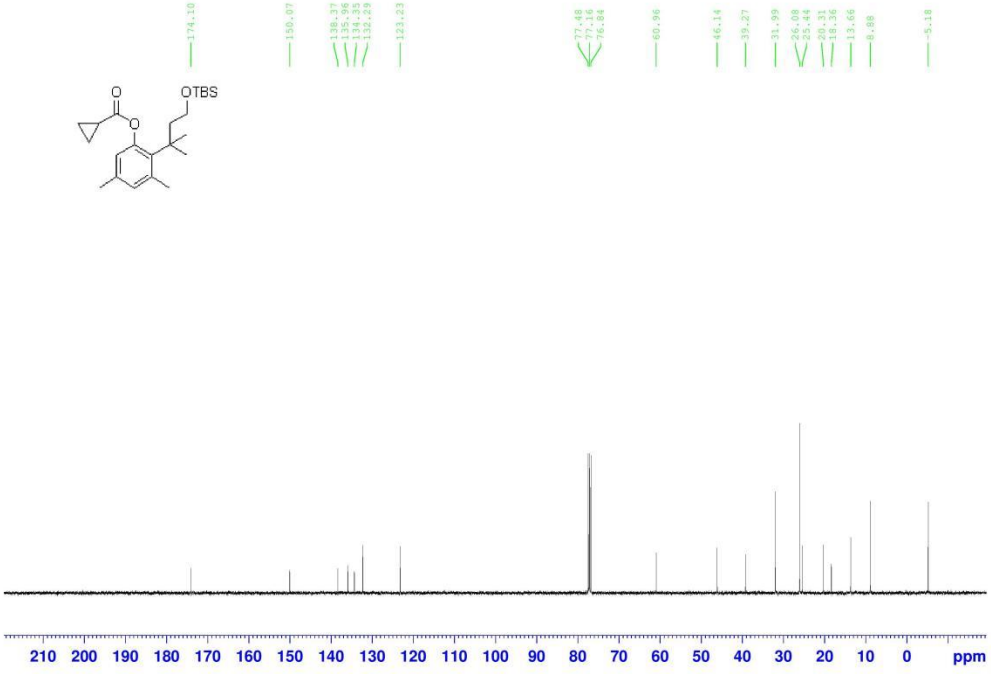
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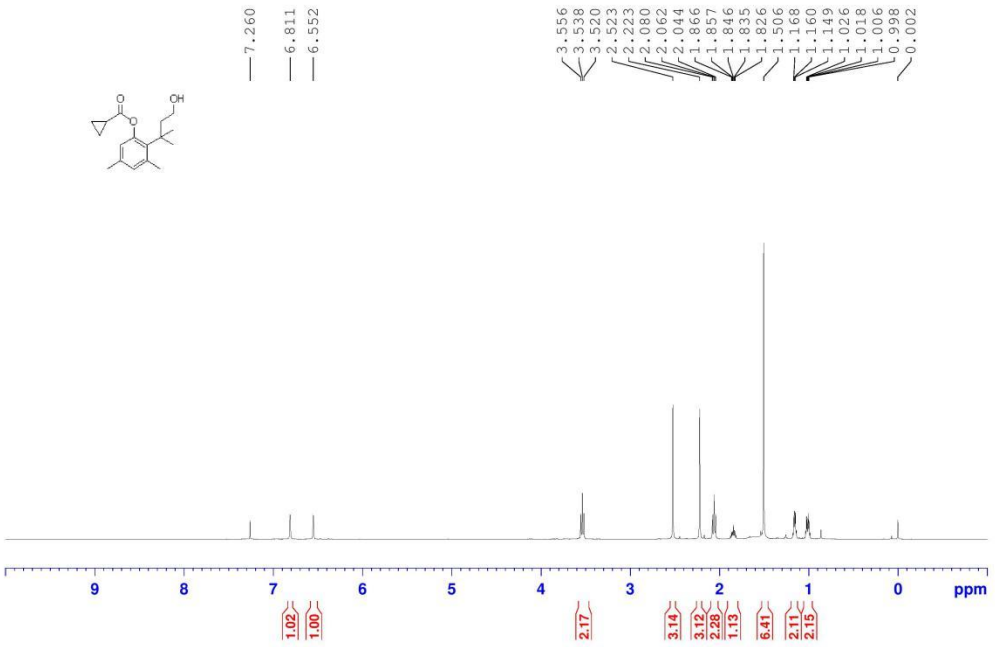
135.96
134.35
132.29
123.23
77.48
77.16
76.84
60.96
46.14
39.27
31.99
26.08
25.44
20.31
19.36
13.66
8.88
-5.18
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm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3.556
3.538
3.520
2.523
2.223
2.080
2.062
2.044
1.866
1.857
1.846
1.835
1.826
1.506
1.168
1.160
1.149
1.026
1.018
1.006
0.998
0.002
9
8
7
6
5
4
3
2
1
0
ppm
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1.00
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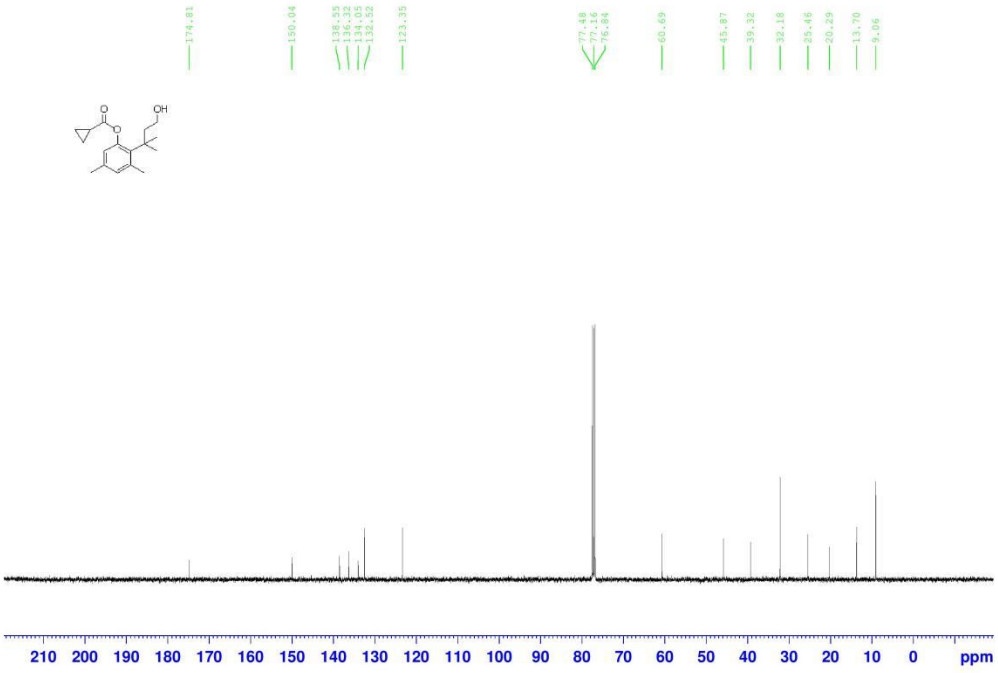
3.12
2.28
1.13
6.41
2.11
2.15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138.55
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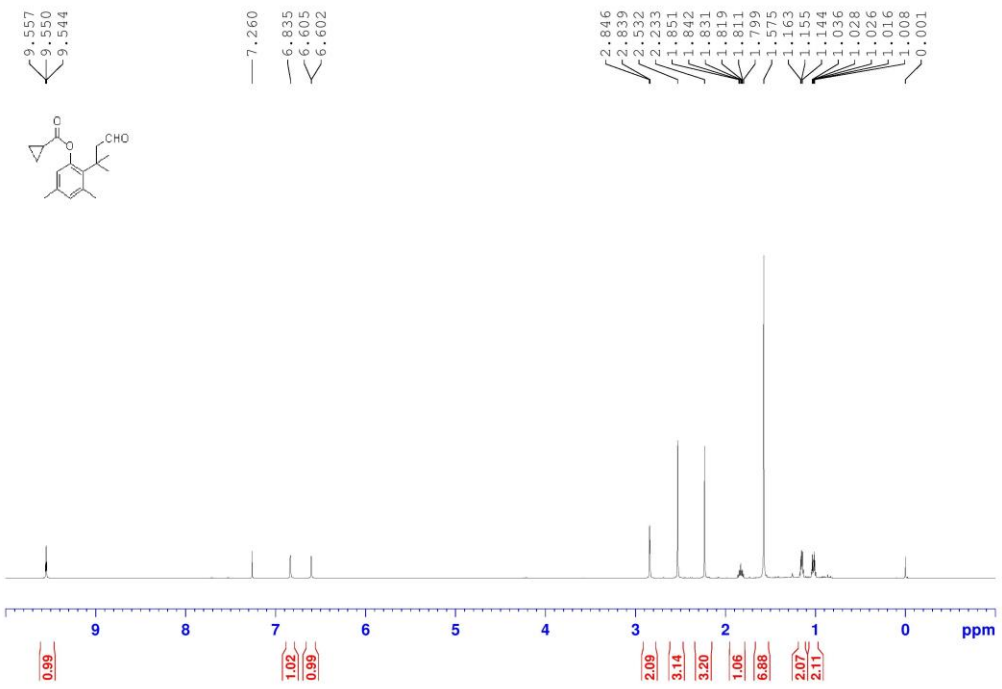
134.05
132.52
123.35
77.48
77.16
76.84
60.69
45.87
39.32
32.18
25.46
20.29
13.70
9.06
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm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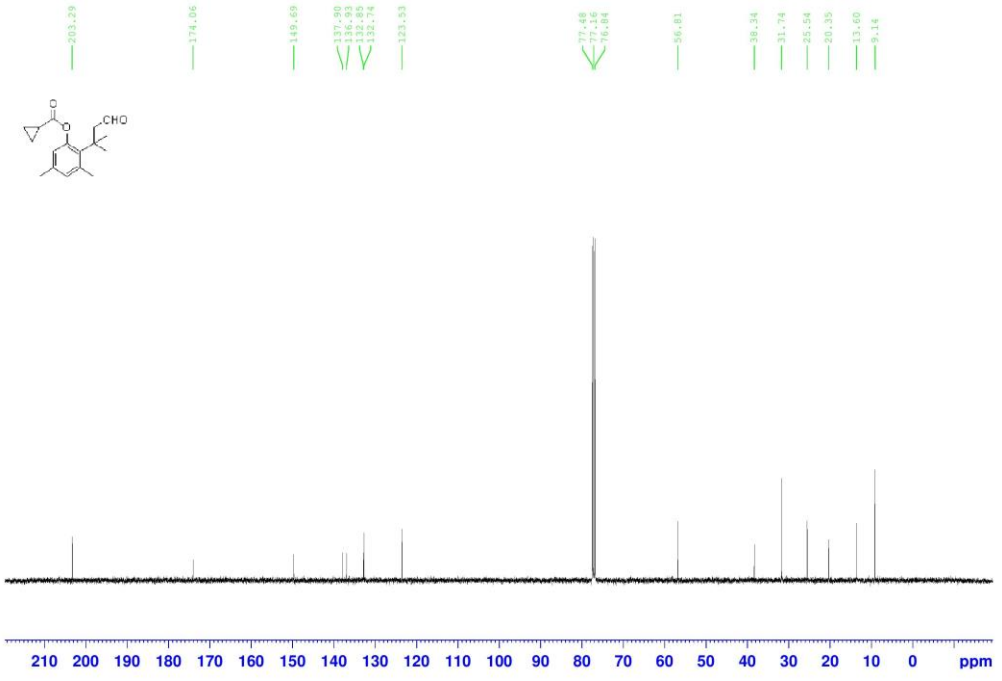
9.544
7.260
6.835
6.605
6.602
2.846
2.839
2.532
2.233
1.851
1.842
1.831
1.819
1.811
1.799
1.575
1.163
1.155
1.144
1.036
1.028
1.026
1.016
1.008
0.001
CHO
9
8
7
6
5
4
3
2
1
0
ppm
0.99
1.02
0.99
2.09
3.14
3.20
1.06
6.88
2.07
2.11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132.85
132.74
123.53
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77.16
76.84
56.81
38.34
31.74
25.54
20.35
13.60
9.14
CHO
210
200
190
180
170
160
150
140
130
120
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100
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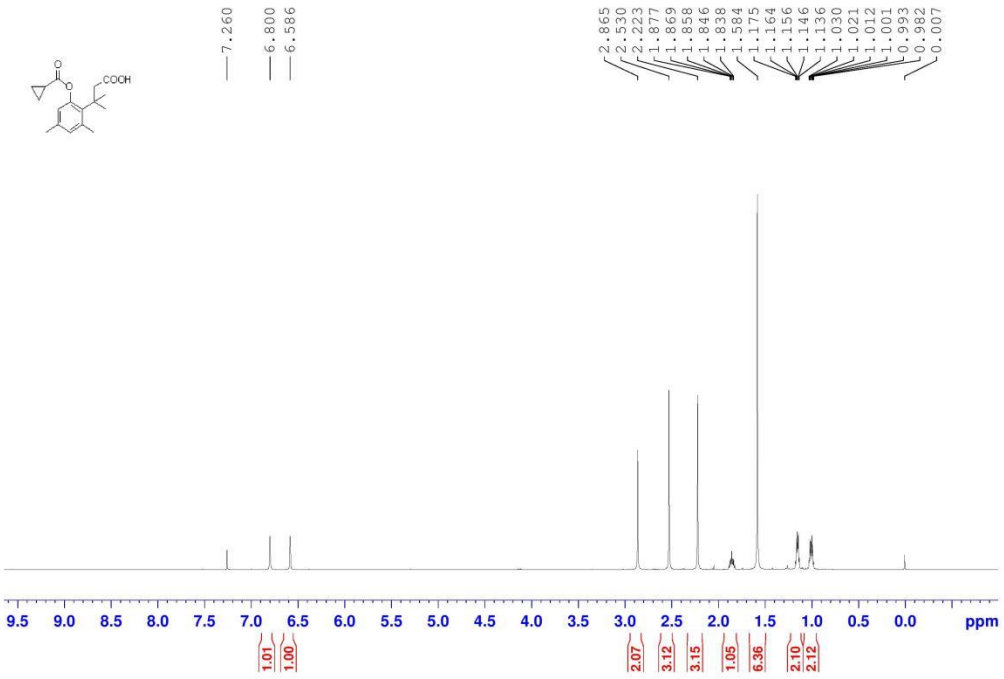
70
60
50
40
30
20
10
0
ppm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2.530
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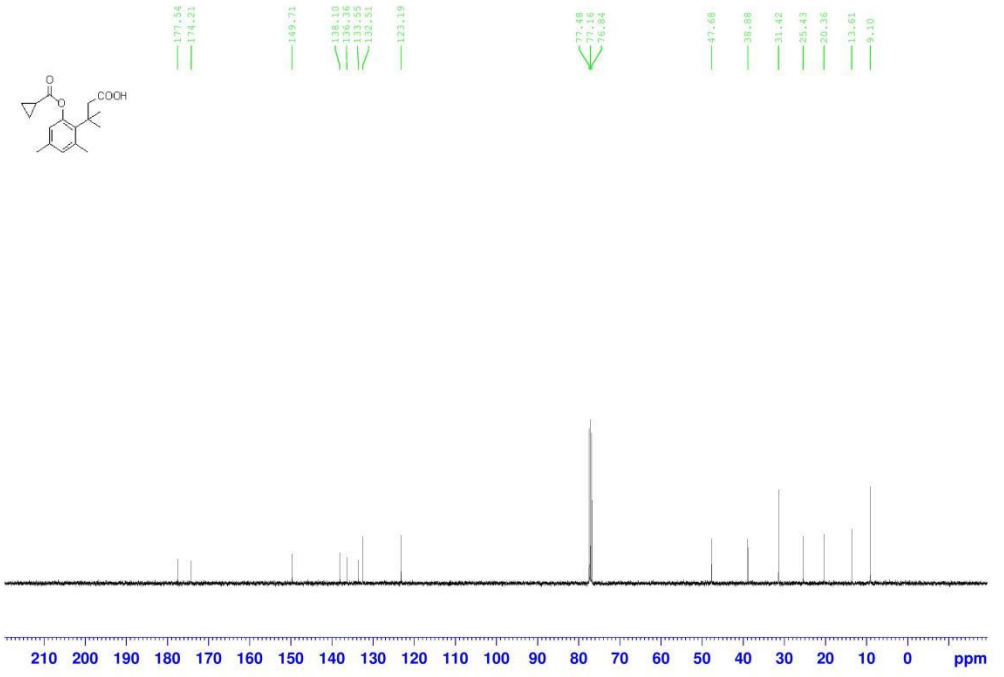
1.877
1.869
1.858
1.846
1.838
1.584
1.175
1.164
1.156
1.146
1.136
1.030
1.021
1.012
1.001
0.993
0.982
0.007
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
1.01
1.00
2.07
3.12
3.15
1.05
6.36
2.10
2.12


COOH
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138.10
136.36
133.55
132.51
123.19
77.48
77.16
76.84
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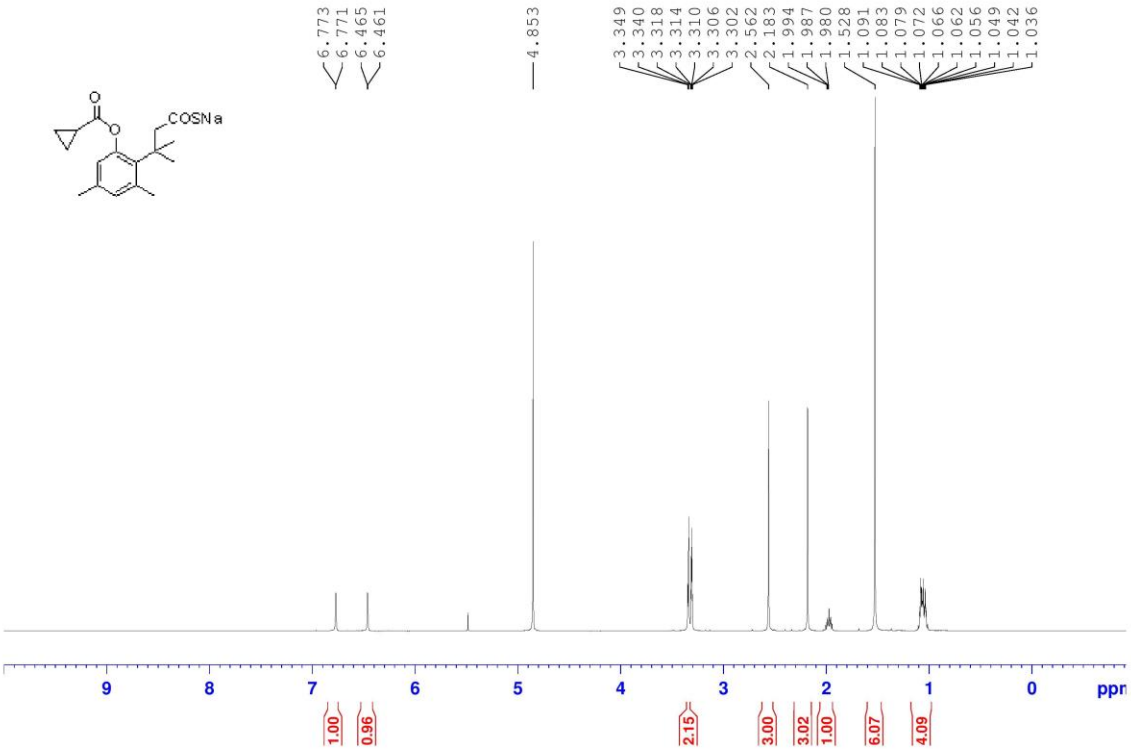
9.10
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm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2.183
1.994
1.987
1.980
1.528
1.091
1.083
1.079
1.072
1.066
1.062
1.056
1.049
1.042
1.036
COSNa
9
8
7
6
5
4
3
2
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ppm
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0.96
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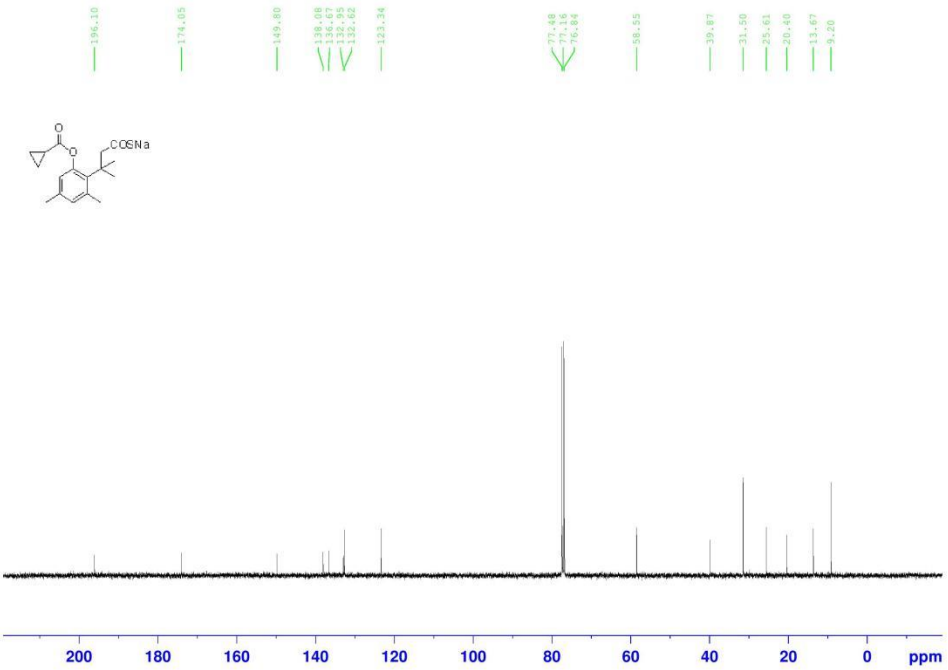
3.00
3.02
1.00
6.07
4.09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132.02
123.34
77.48
77.16
76.84
58.55
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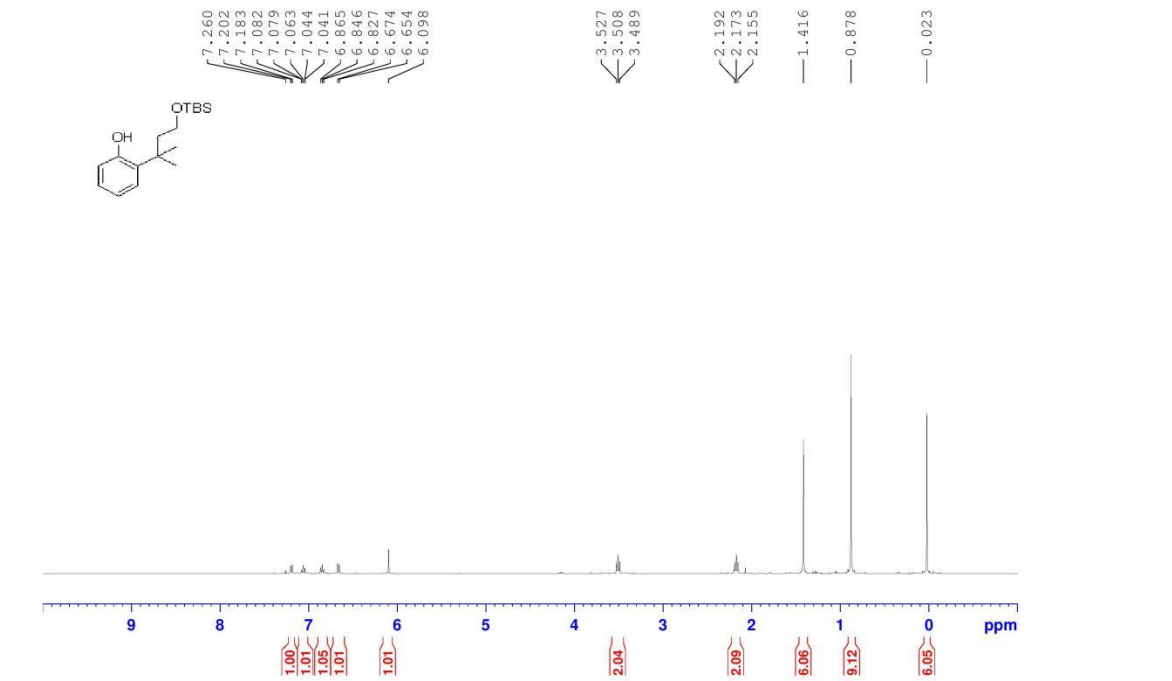
31.50
25.61
20.40
13.67
9.20
COSNa
200
180
160
140
120
100
80
60
40
20
0
ppm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7.079
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7.044
7.041
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6.846
6.827
6.674
6.654
6.098
3.527
3.508
3.489
2.192
2.173
2.155
1.416
0.878
0.023
OTBS
OH
9
8
7
6
5
4
3
2
1
0
ppm
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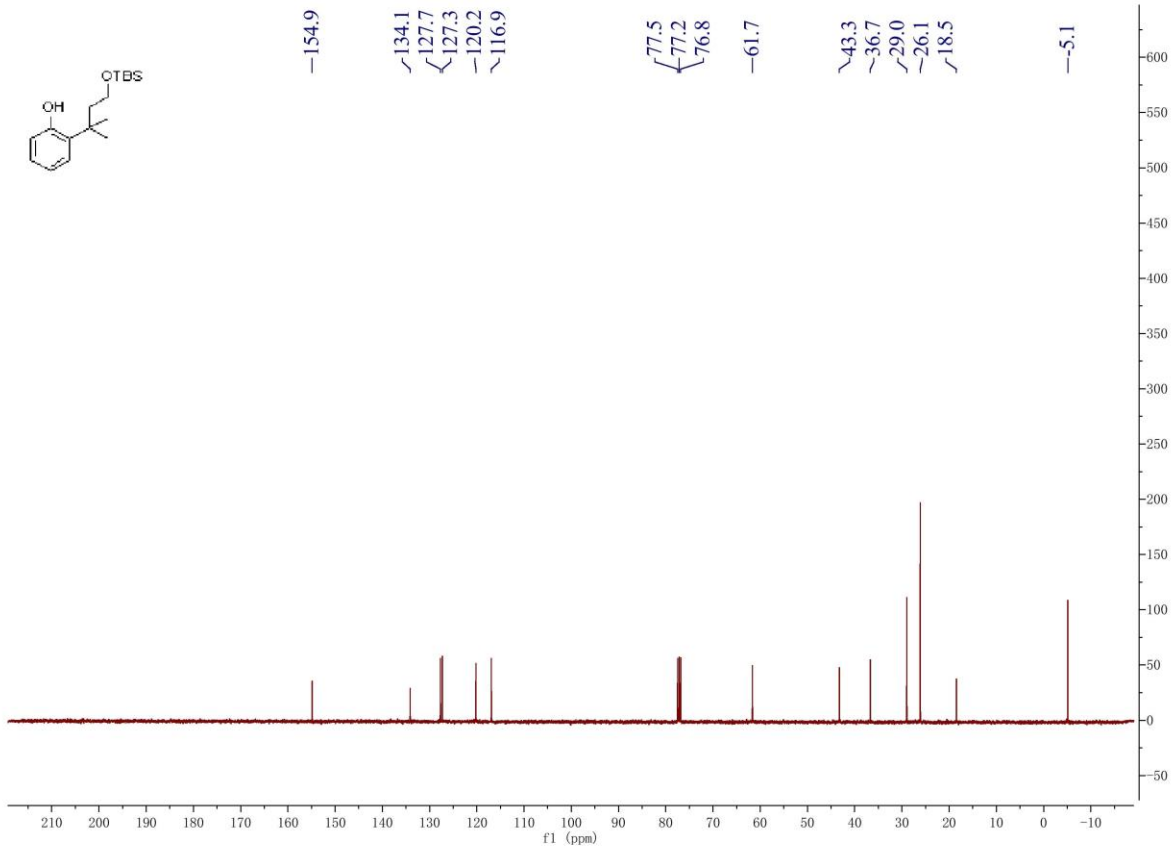
1.01
1.05
1.01
1.01
2.04
2.09
6.06
9.12
6.05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127.7
127.3
120.2
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77.5
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76.8
61.7
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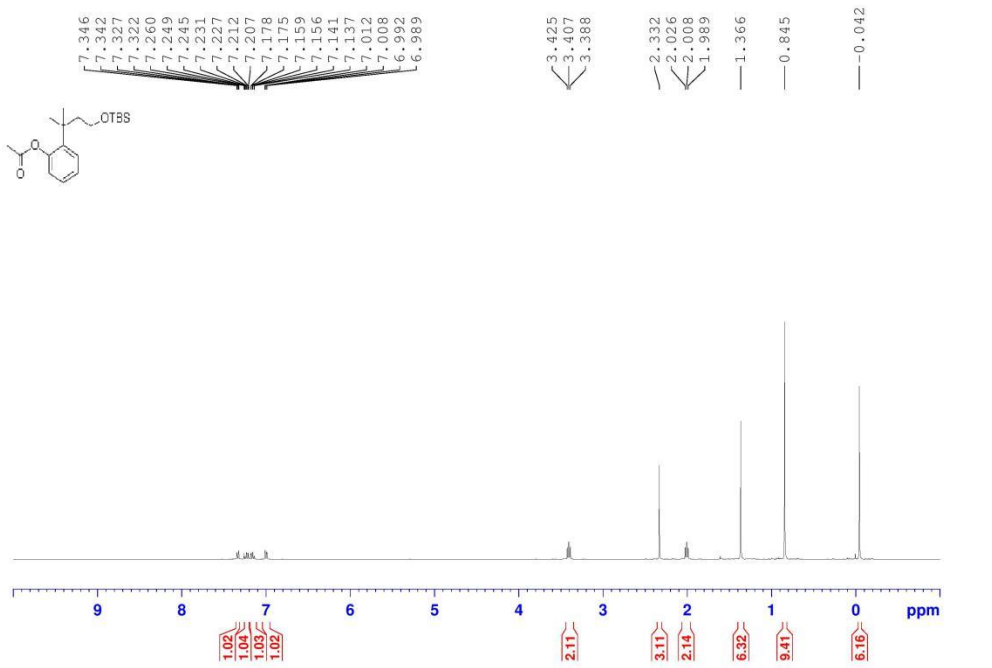
26.1
18.5
-5.1
OTBS
OH
f1 (ppm)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7.178
7.175
7.159
7.156
7.141
7.137
7.012
7.008
6.992
6.989
3.425
3.407
3.388
2.332
2.026
2.008
1.989
1.366
0.845
-0.042
OTBS
9
8
7
6
5
4
3
2
1
0
ppm
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1.04
1.03
1.02
2.11
3.11
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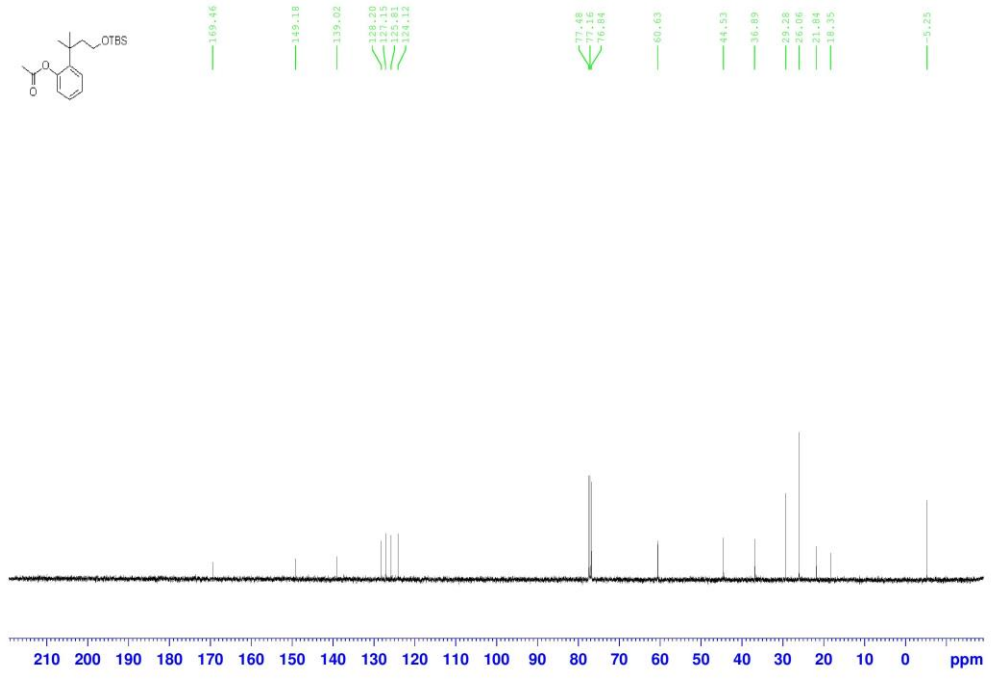
OTBS
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77.48
77.16
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60.63
44.53
36.89
29.28
26.06
21.84
18.35
-5.25
210
200
190
180
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140
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100
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80
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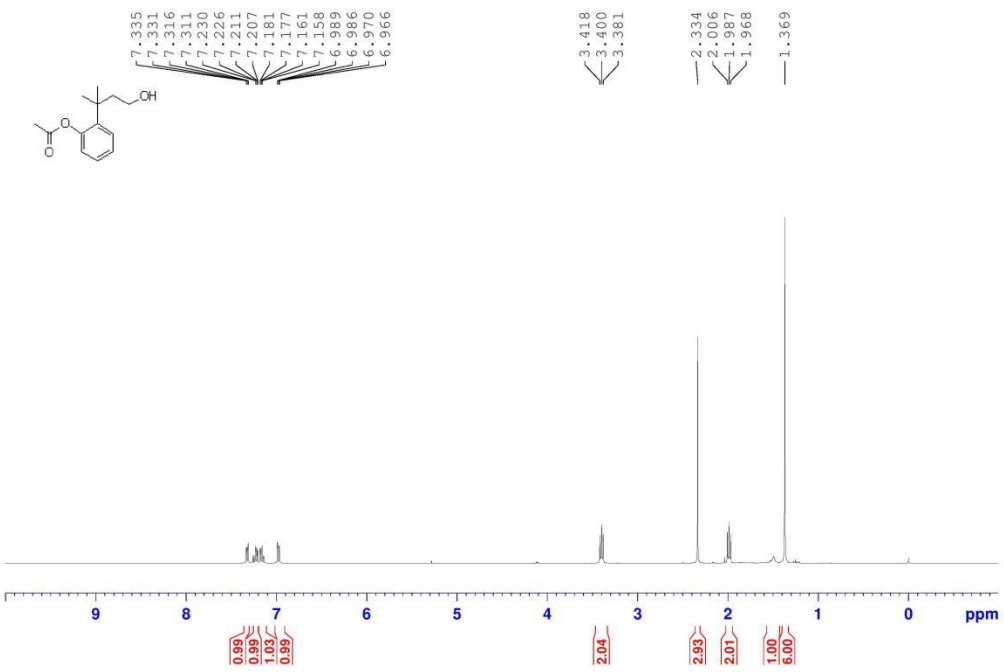
60
50
40
30
20
10
0
ppm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7.207
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6.986
6.970
6.966
3.418
3.400
3.381
2.334
2.006
1.987
1.968
1.369
OH
O
O
9
8
7
6
5
4
3
2
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ppm
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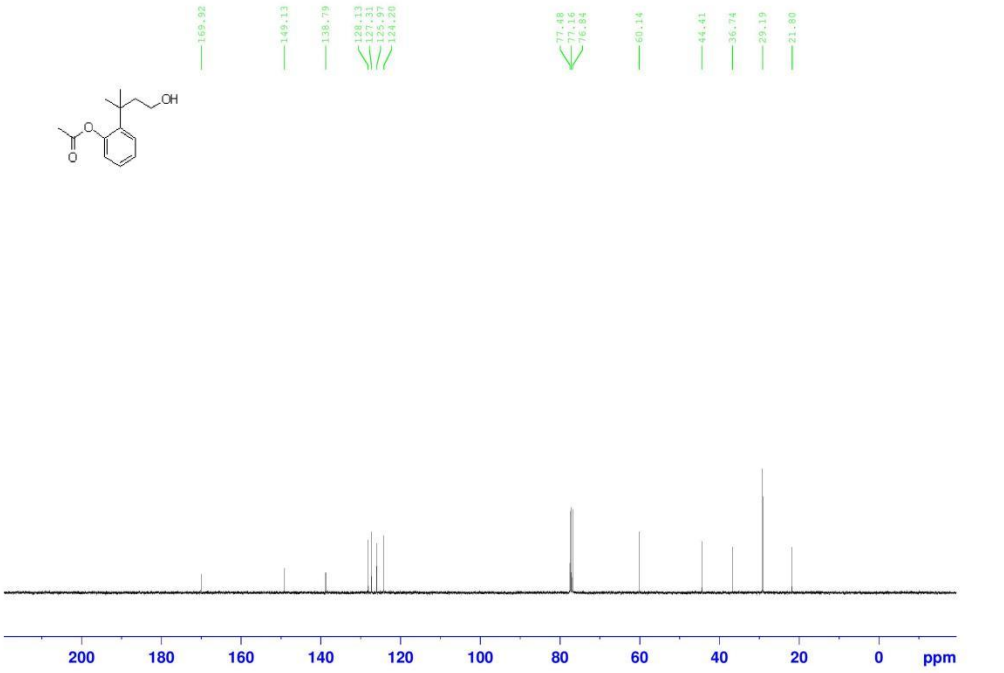
0.99
1.03
0.99
2.04
2.93
2.01
1.00
6.00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125.97
124.20
77.48
77.16
76.84
60.14
44.41
36.74
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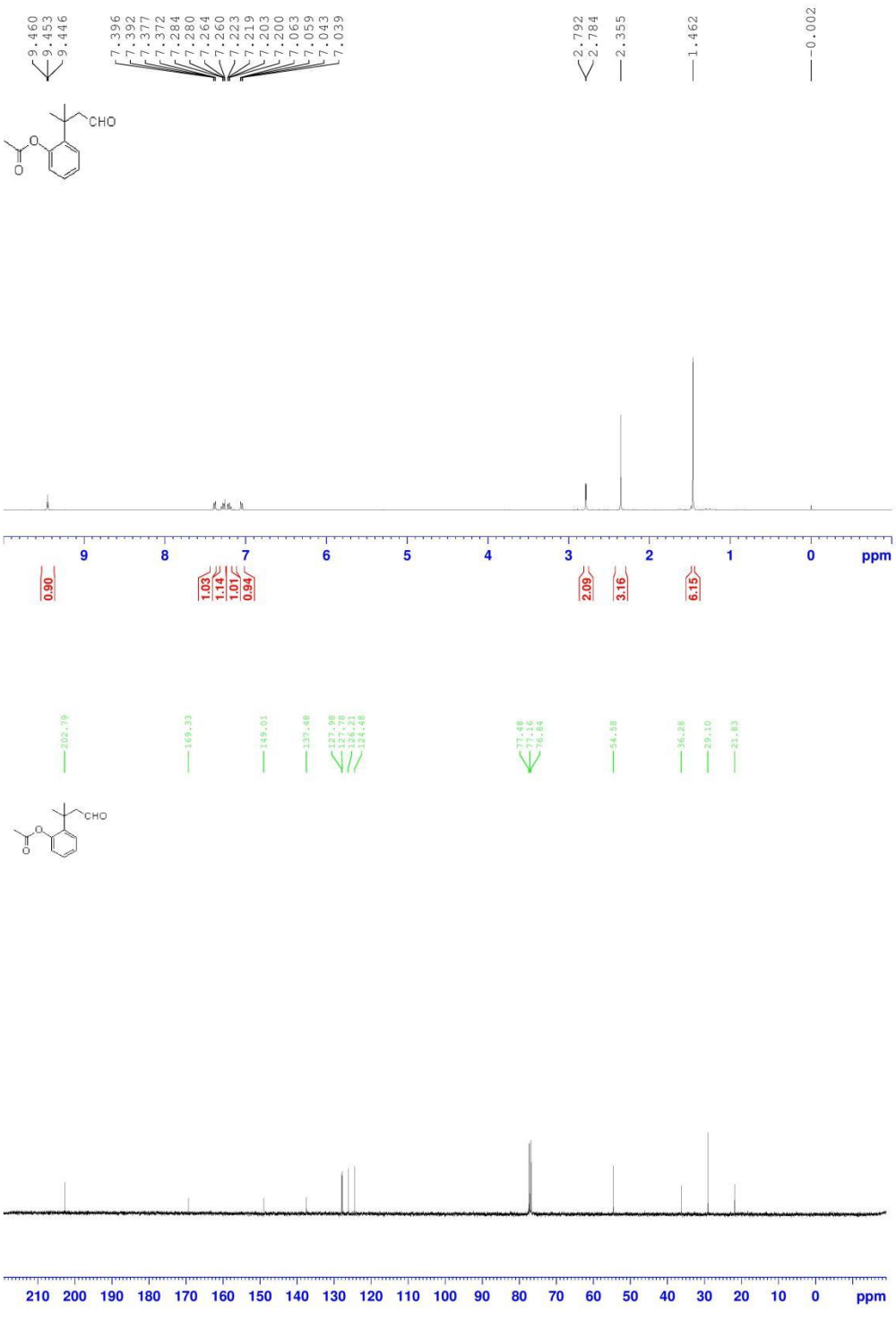
OH
O
O
200
180
160
140
120
100
80
60
40
20
0
ppm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7.392
7.377
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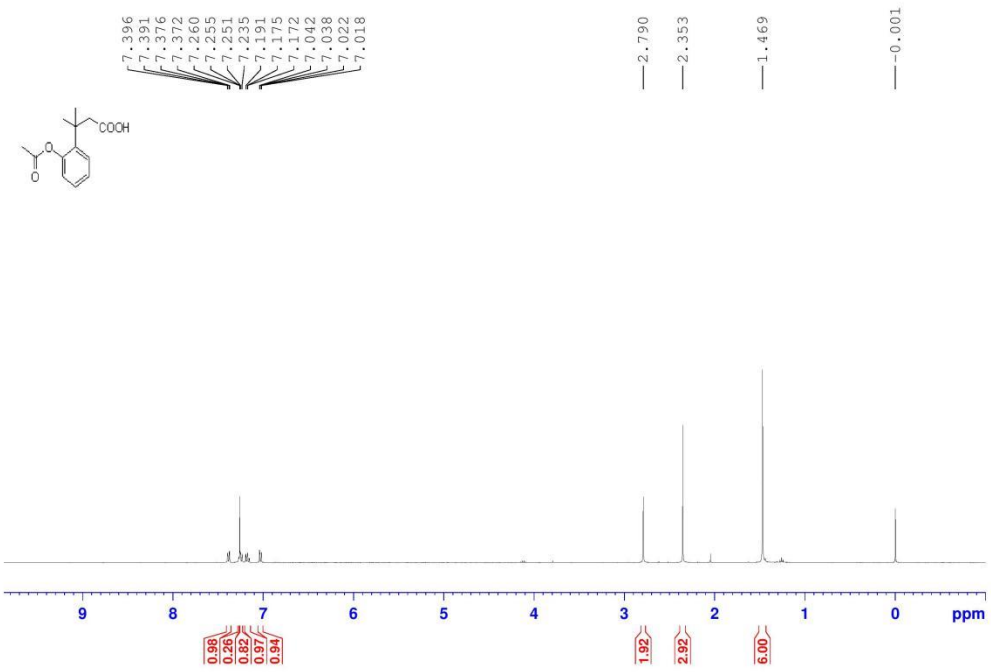
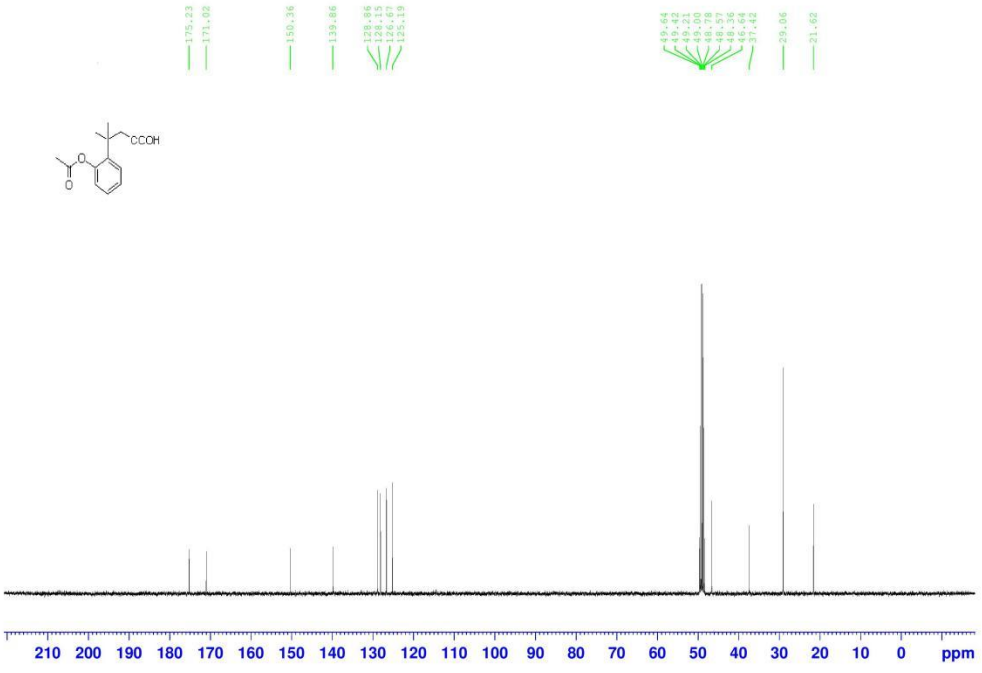
7.284
7.280
7.264
7.260
7.223
7.219
7.203
7.200
7.063
7.059
7.043
7.039
2.792
2.784
2.355
1.462
0.002
CHO
O
O
9
8
7
6
5
4
3
2
1
0
ppm
0.90
1.03
1.14
1.01
0.94
2.09
3.16
6.15
202.79
169.33
149.01
137.48
127.98
127.78
126.21
124.48
77.48
77.16
76.84
54.58
36.28
29.10
21.83
CHO
O
O
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm


7.396
7.391
7.376
7.372
7.260
7.255
7.251
7.235
7.191
7.175
7.172
7.042
7.038
7.022
7.018
2.790
2.353
1.469
-0.001
COOH
9
8
7
6
5
4
3
2
1
0
ppm
0.98
0.26
0.82
0.97
0.94
1.92
2.92
6.00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171.02
150.36
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128.15
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125.19
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48.36
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37.42
29.06
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CCOH
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200
190
180
170
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150
140
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90
80
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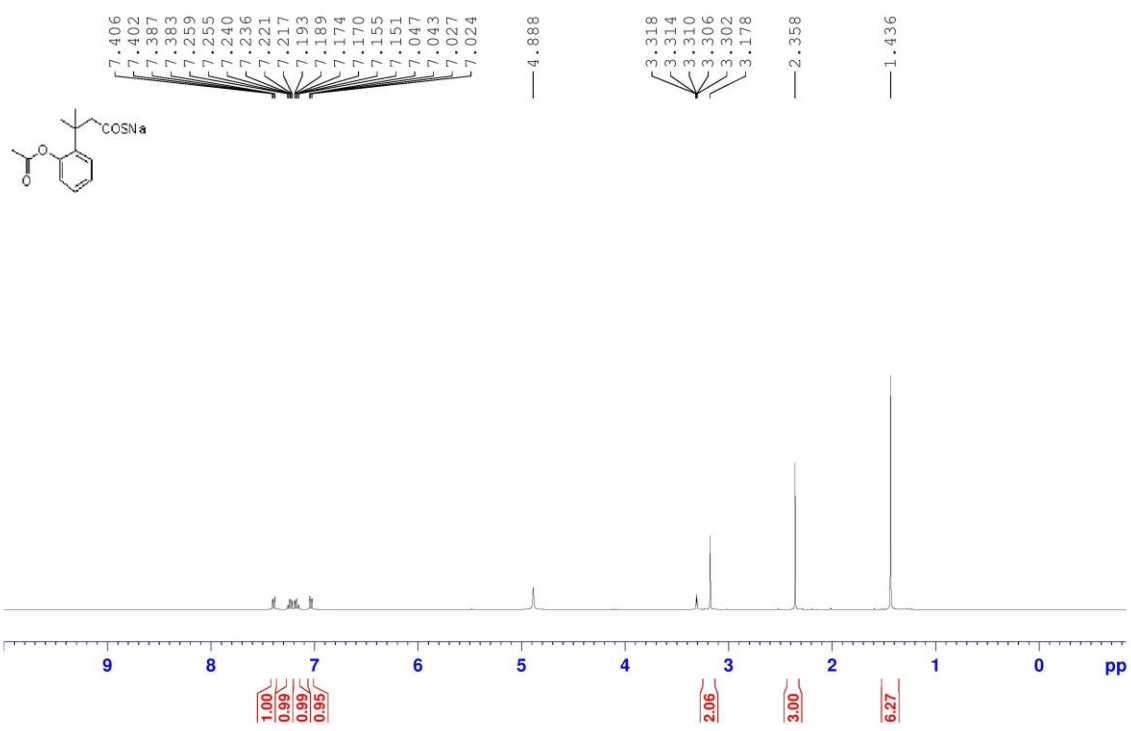
60
50
40
30
20
10
0
ppm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7.221
7.217
7.193
7.189
7.174
7.170
7.155
7.151
7.047
7.043
7.027
7.024
4.888
3.318
3.314
3.310
3.306
3.302
3.178
2.358
1.436
COSNa
9
8
7
6
5
4
3
2
1
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pp
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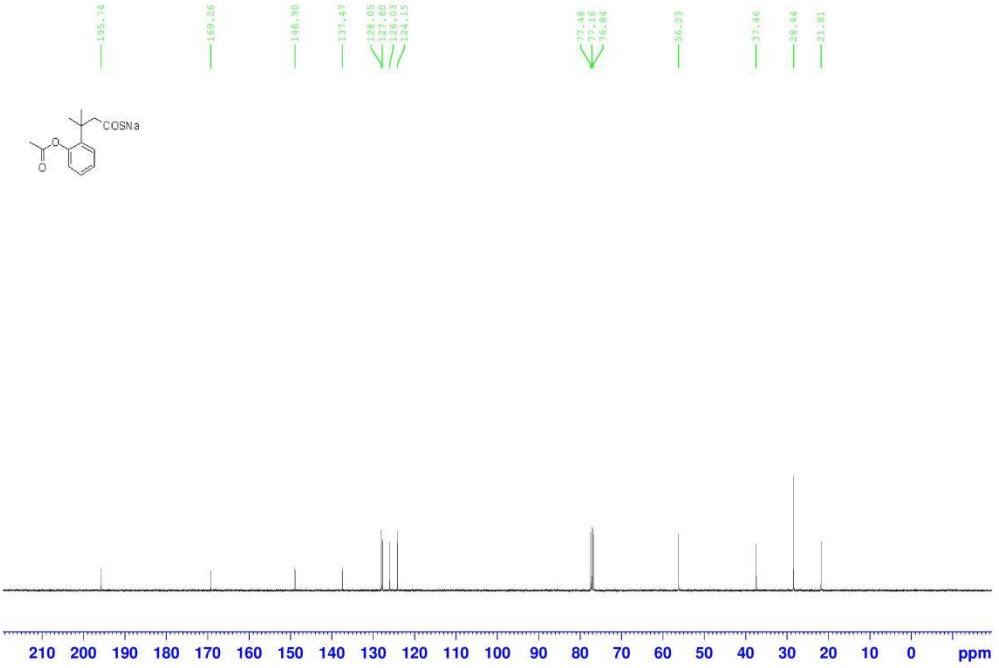
0.99
0.99
0.95
2.06
3.00
6.27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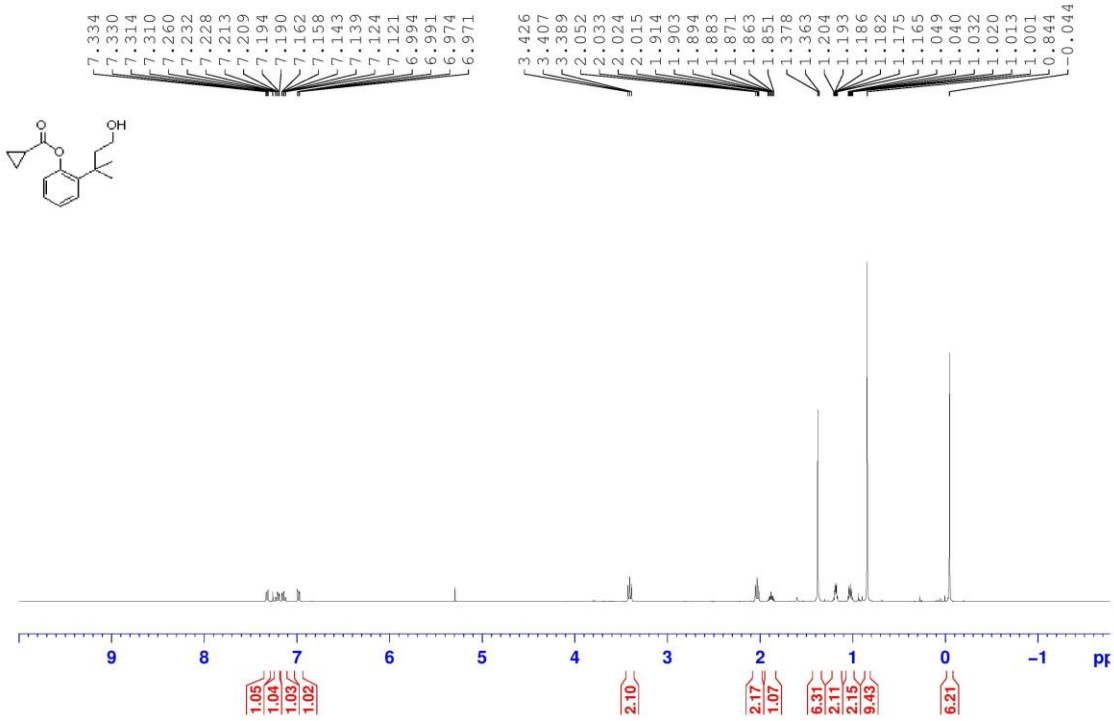
127.80
126.03
124.15
77.48
77.16
76.84
56.23
37.46
28.44
21.81
COSNa
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm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7.232
7.228
7.213
7.209
7.194
7.190
7.162
7.158
7.143
7.139
7.124
7.121
6.994
6.991
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2.015
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1.863
1.851
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1.182
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1.049
1.040
1.032
1.020
1.013
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-0.044
OH
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5
4
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−1
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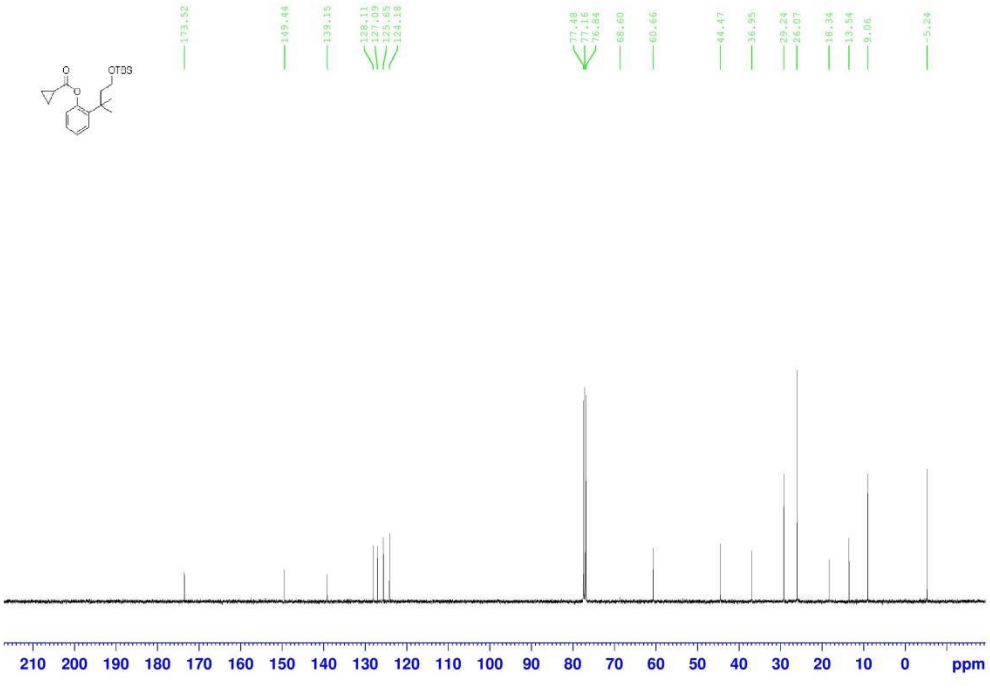
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44.47
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29.24
26.07
18.34
13.54
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OTBS
210
200
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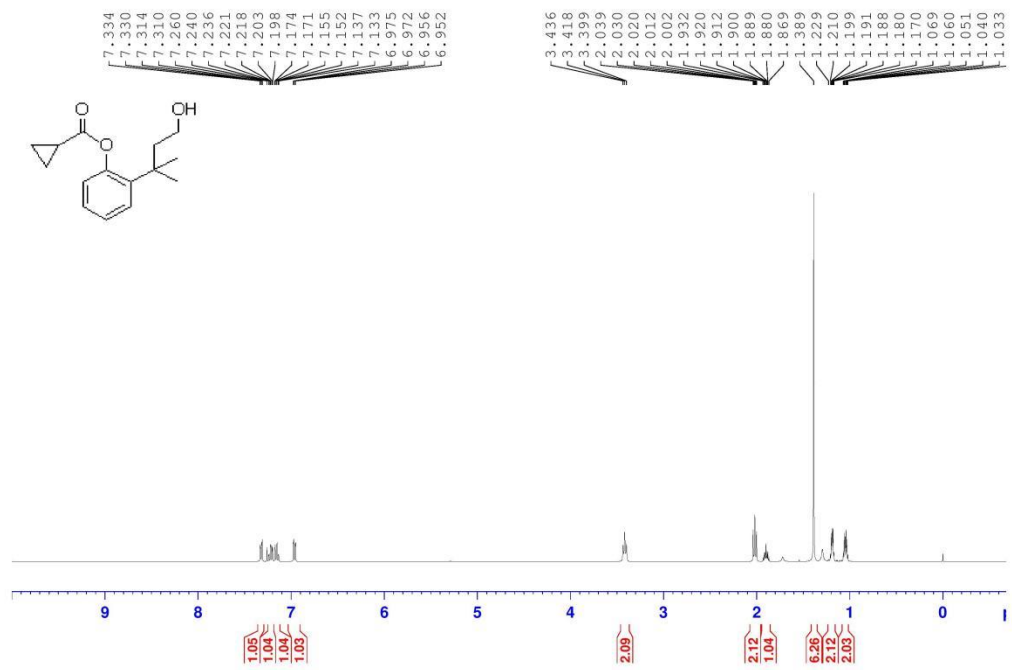
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7.171
7.155
7.152
7.137
7.133
6.975
6.972
6.956
6.952
3.436
3.418
3.399
2.039
2.030
2.020
2.012
2.002
1.932
1.920
1.912
1.900
1.889
1.880
1.869
1.389
1.229
1.210
1.199
1.191
1.188
1.180
1.170
1.069
1.060
1.051
1.040
1.033
OH
O
O
9
8
7
6
5
4
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1.03
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2.12
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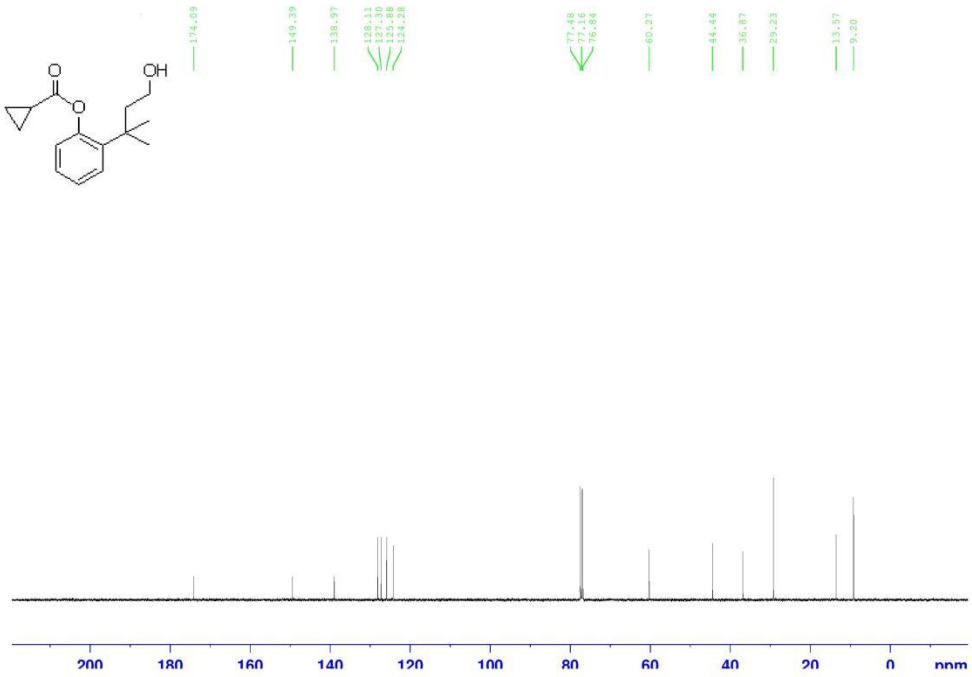
2.12
2.03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60.27
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O
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120
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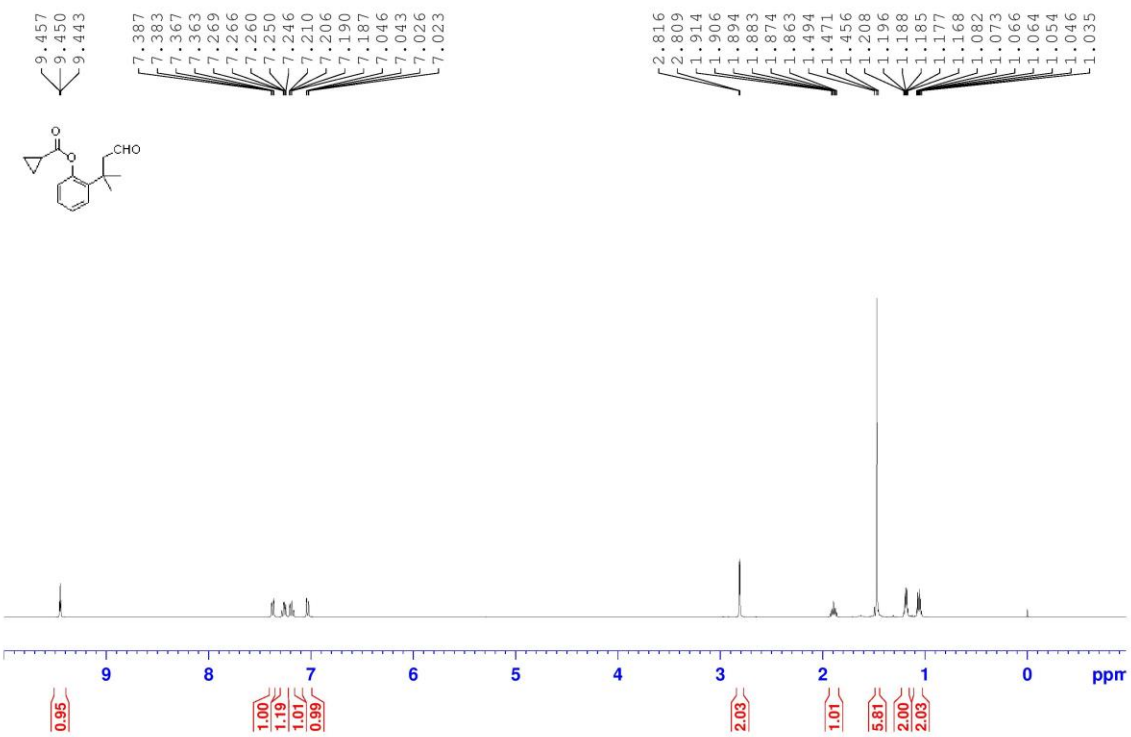
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7.250
7.246
7.210
7.206
7.190
7.187
7.046
7.043
7.026
7.023
2.816
2.809
1.914
1.906
1.894
1.883
1.874
1.863
1.494
1.471
1.456
1.208
1.196
1.188
1.185
1.177
1.168
1.082
1.073
1.066
1.064
1.054
1.046
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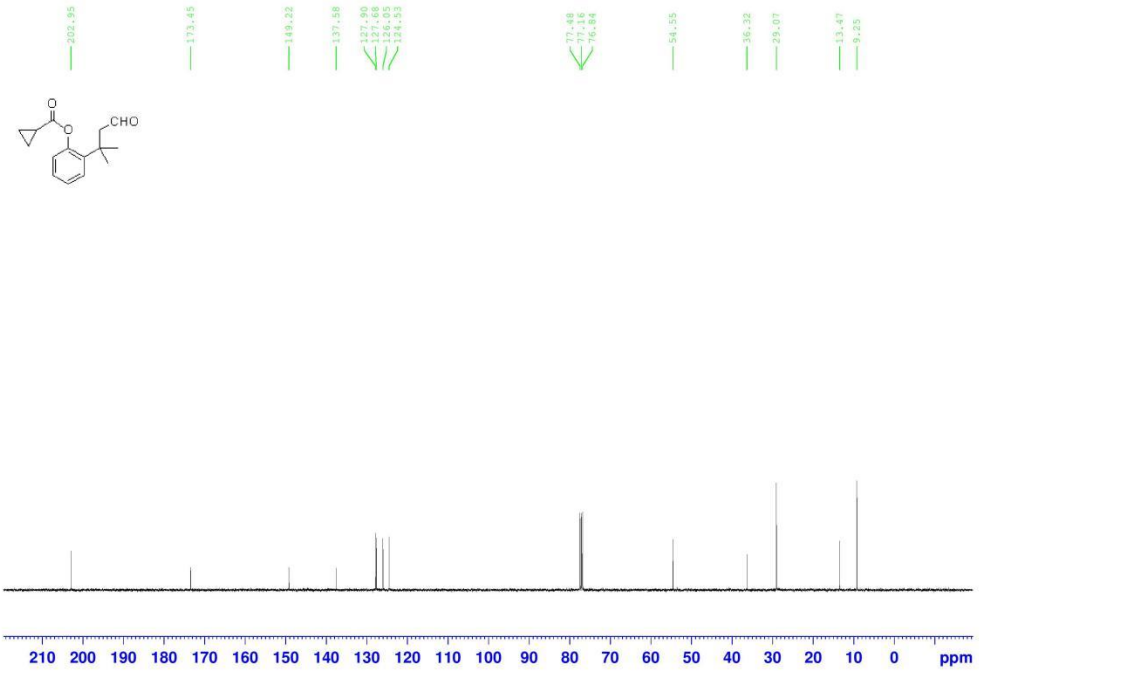
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76.84
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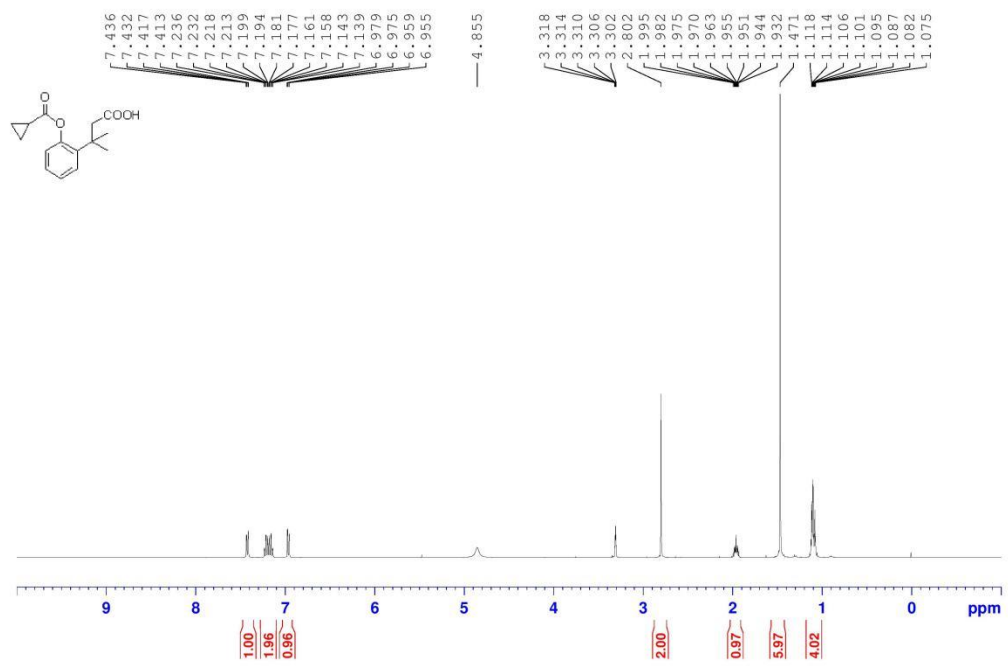
29.07
13.47
9.25
CHO
ppm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7.213
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7.181
7.177
7.161
7.158
7.143
7.139
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6.975
6.959
6.955
4.855
3.318
3.314
3.310
3.306
3.302
2.802
1.995
1.982
1.975
1.970
1.963
1.955
1.951
1.944
1.932
1.471
1.118
1.114
1.106
1.101
1.095
1.087
1.082
1.075
COOH
9
8
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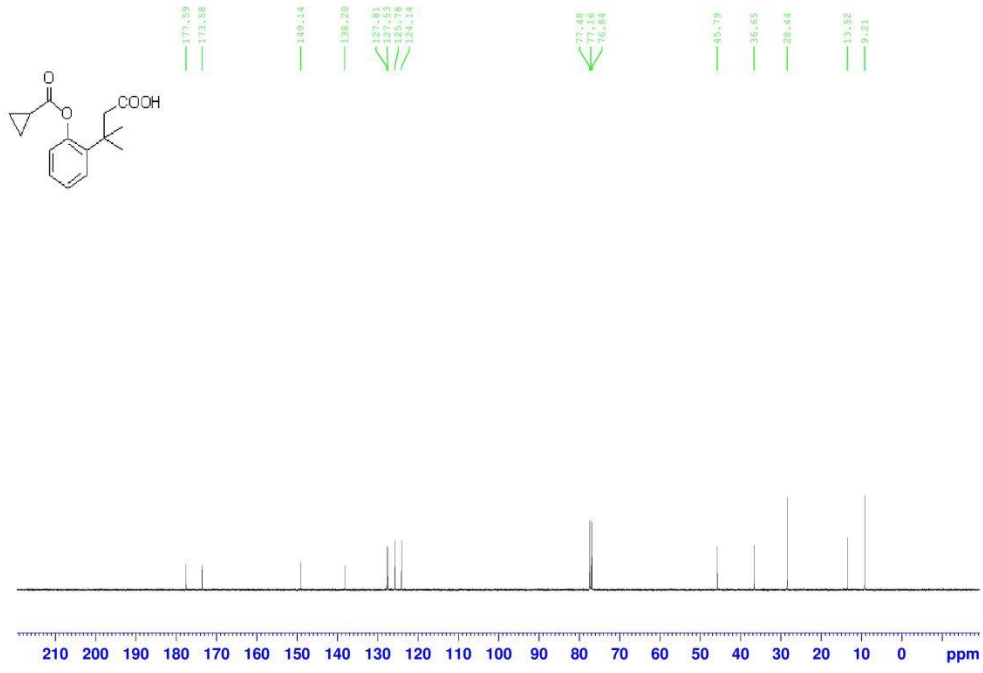
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124.14
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77.16
76.84
45.79
36.65
28.44
13.52
9.21
COOH
210
200
190
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120
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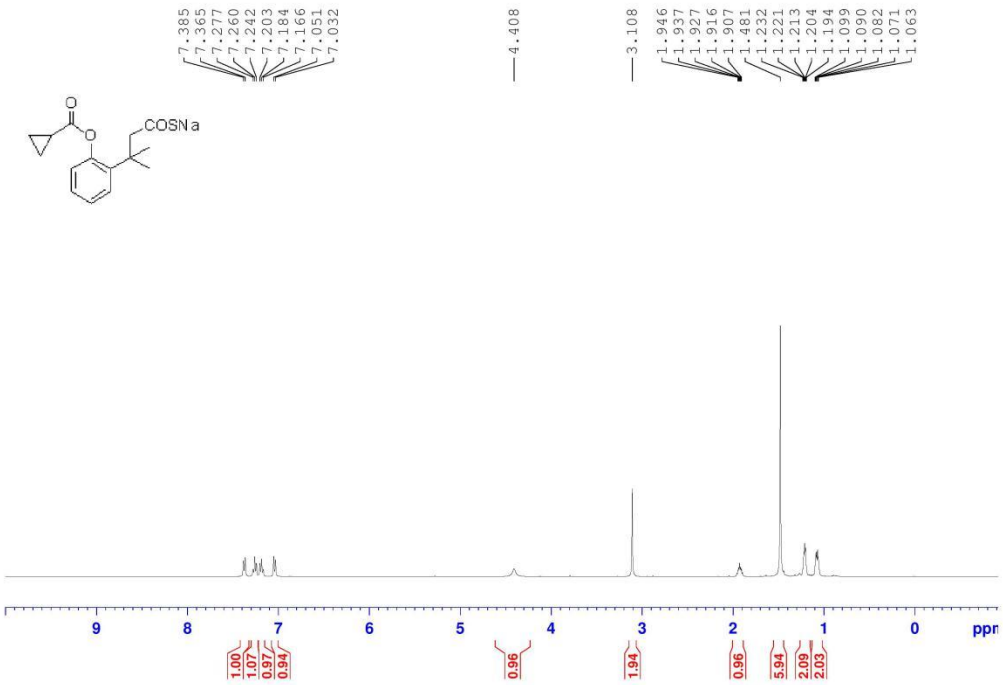
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7.166
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7.032
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3.108
1.946
1.937
1.927
1.916
1.907
1.481
1.232
1.221
1.213
1.204
1.194
1.099
1.090
1.082
1.071
1.063
COSNa
9
8
7
6
5
4
3
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1.94
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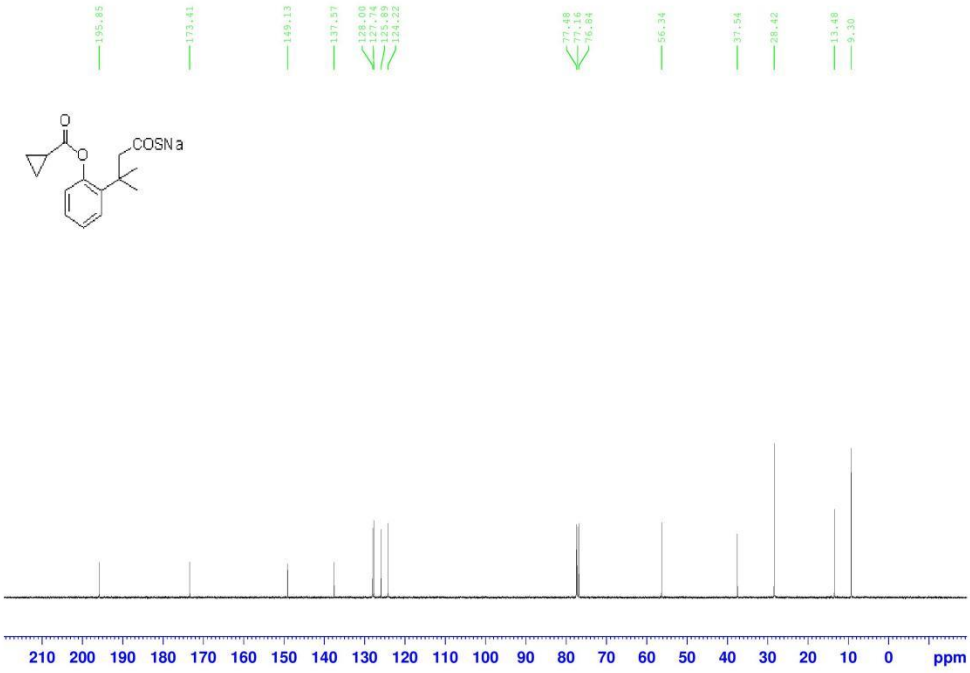
2.03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77.16
76.84
56.34
37.54
28.42
13.49
9.30
COSNa
210
200
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140
130
120
110
100
90
80
70
60
50
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30
20
10
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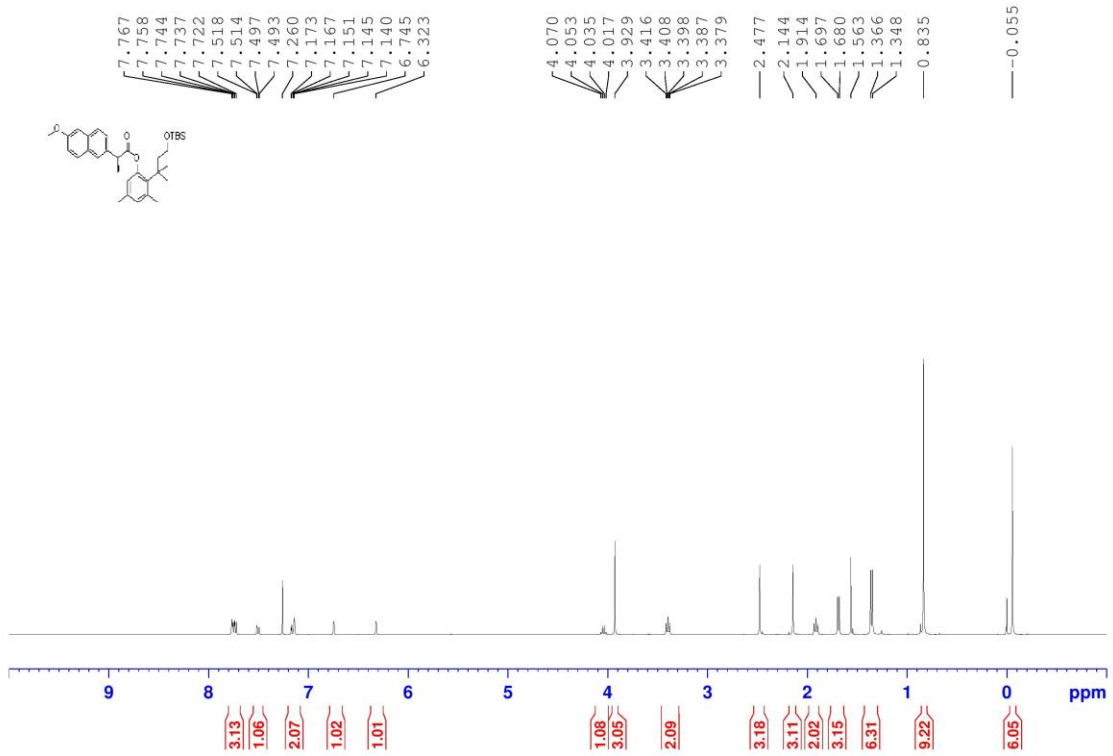
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7.145
7.140
6.745
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4.070
4.053
4.035
4.017
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3.408
3.398
3.387
3.379
2.477
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-0.055
OTBS
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8
7
6
5
4
3
2
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0
ppm
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2.07
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2.02
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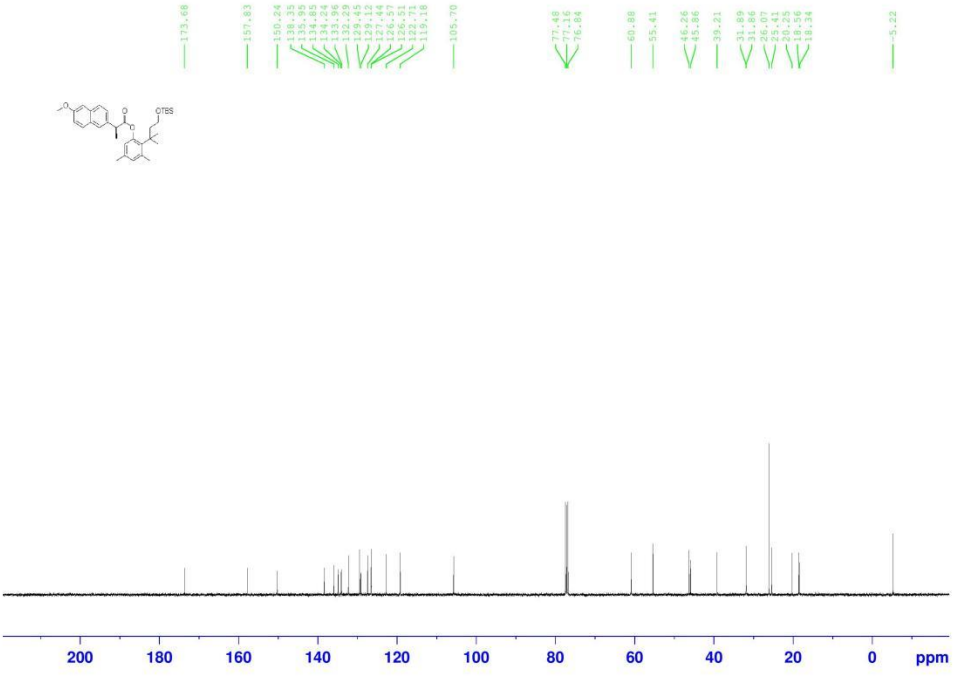
6.05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122.71
119.18
105.70
77.48
77.15
76.84
60.88
55.44
46.26
45.86
39.21
31.89
31.66
26.07
25.41
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-5.22
OTBS
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120
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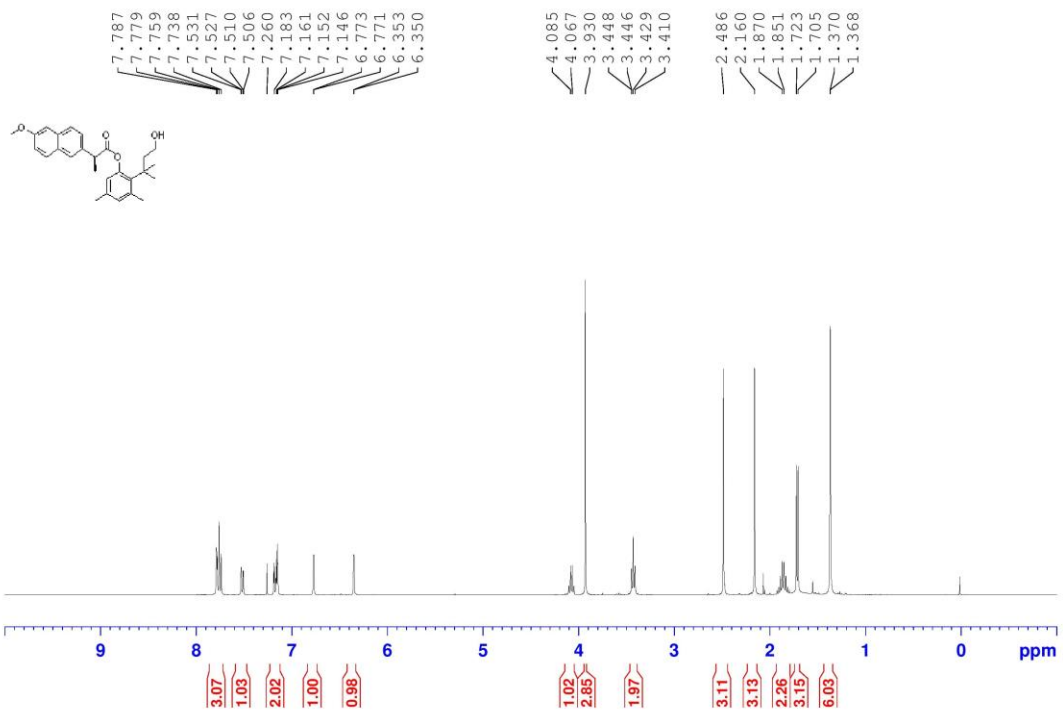
20
0
ppm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6.771
6.353
6.350
4.085
4.067
3.930
3.448
3.446
3.429
3.410
2.486
2.160
1.870
1.851
1.723
1.705
1.370
1.368
OH
9
8
7
6
5
4
3
2
1
0
ppm
3.07
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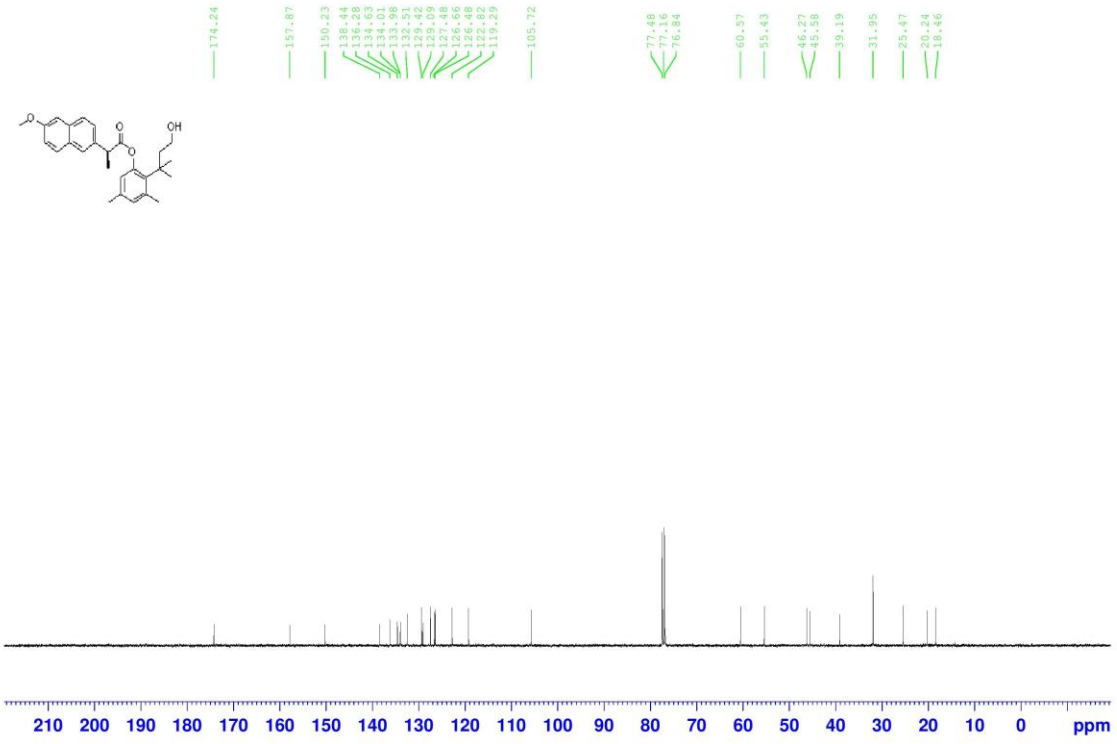
6.03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127.48
126.66
126.48
122.82
119.29
105.72
77.48
77.16
76.84
60.57
55.43
46.27
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19.46
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OH
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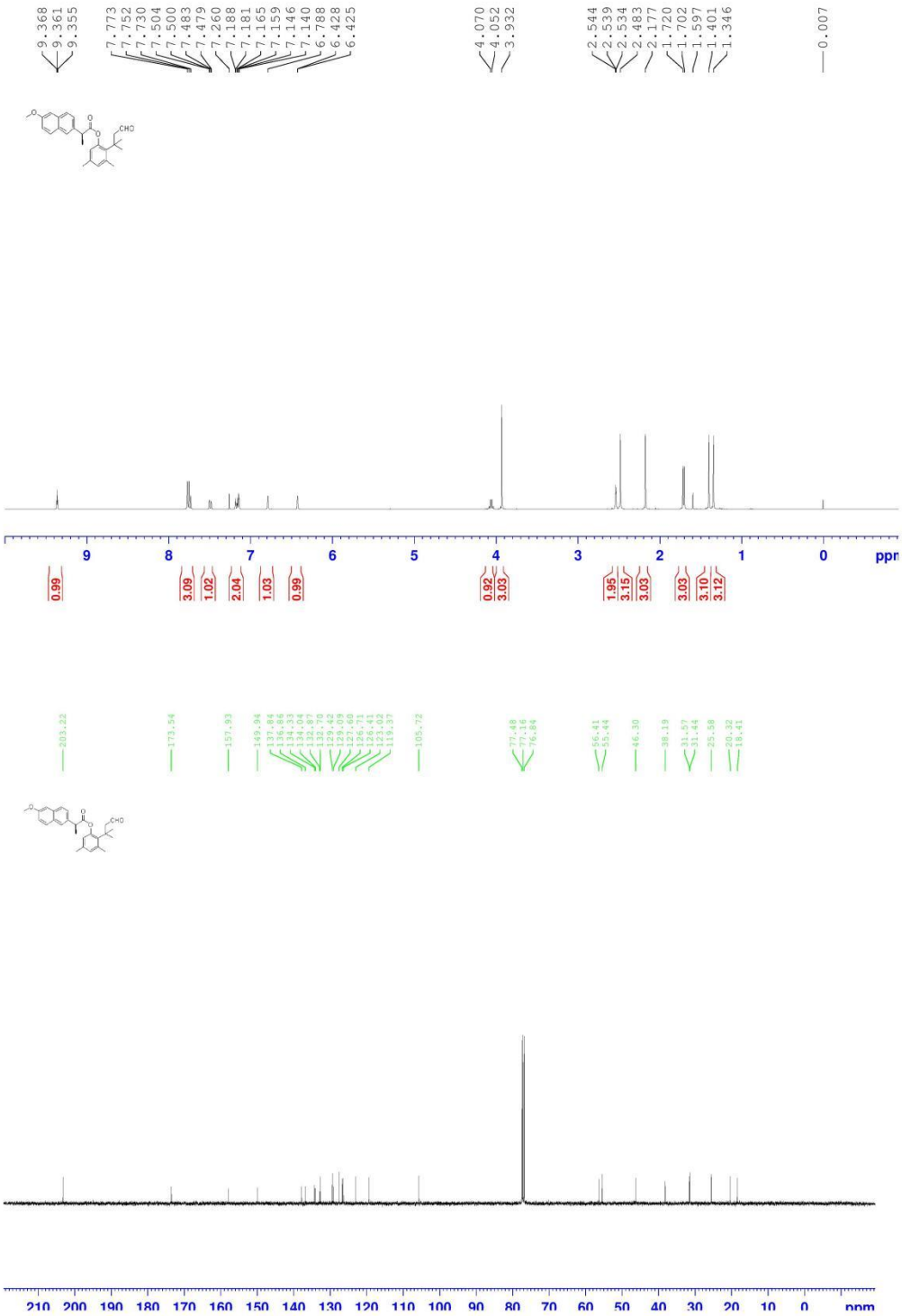
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CHO
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ppm
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1.95
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3.03
3.03
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3.12
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77.48
77.16
76.84
56.41
55.44
46.30
38.19
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31.44
25.58
20.32
18.41
CHO
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200
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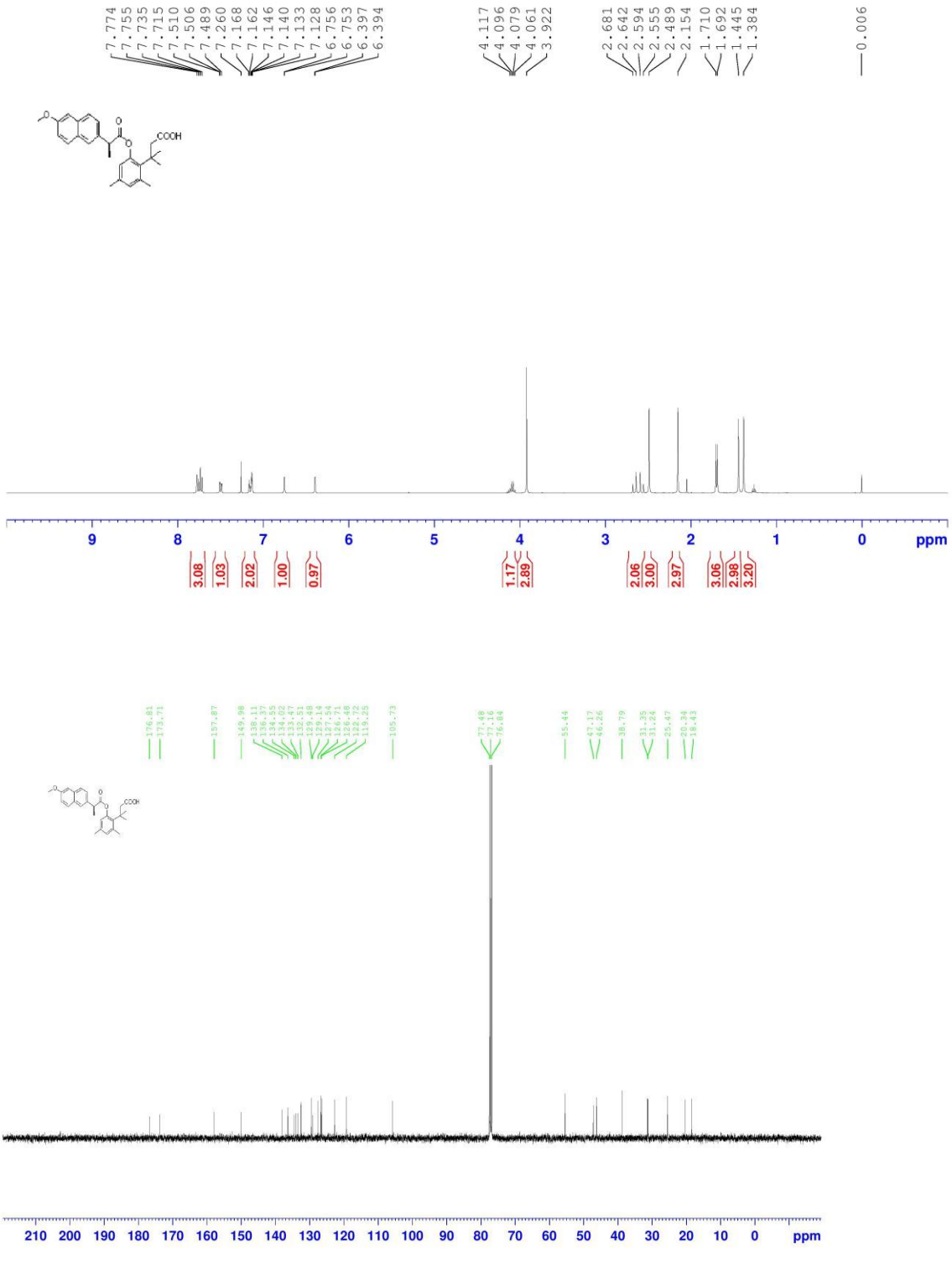
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4.061
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7
6
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3
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2.02
1.00
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3.00
2.97
3.06
2.98
3.20
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125.72
119.26
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77.16
76.84
55.44
47.17
46.26
38.79
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31.24
25.47
20.34
18.43
210
200
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80
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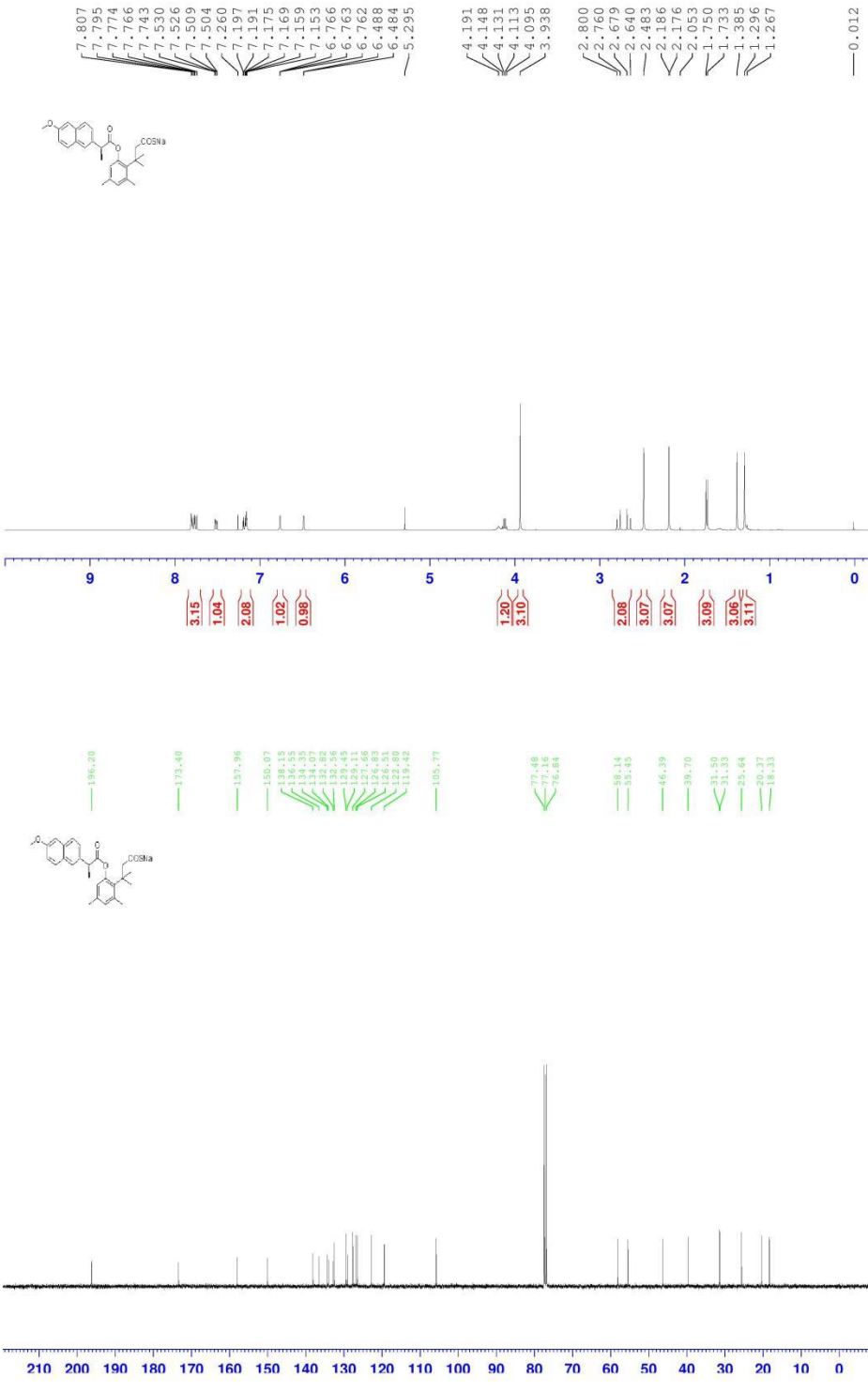
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6.762
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4.131
4.113
4.095
3.938
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2.053
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1.267
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COSNa
9
8
7
6
5
4
3
2
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ppm
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1.04
2.08
1.02
0.98
1.20
3.10
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3.07
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3.06
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76.84
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55.45
46.39
39.70
31.50
31.33
25.64
20.37
18.33
COSNa
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm