Electronic Supporting Information

Chemoenzymatic Total Synthesis of Nodulones C & D using Naphthol Reductase of *Magnaporthe grisea*

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I. General Remarks

All commercial reagents were obtained from Sigma-Aldrich Chemical Co. and Sisco Research Laboratories, India. Reactions were monitored by thin-layer chromatography (TLC, 0.25 mm E. Merck silica gel plates, $60F_{254}$) and the plates were visualized by using UV light. Column chromatography was performed on silica gel 60–120/100-200/230–400 mesh obtained from S. D. Fine Chemical Co., India. Yields refer to chromatographically pure materials; conversions were calculated from the ^{1}H NMR spectra of the crude products. ^{1}H NMR spectra were recorded on Bruker 400 Ultra Shield instruments using deuterated solvents. Proton coupling constants (*J*) are reported as absolute values in Hz. ^{13}C NMR spectra were recorded on Bruker 400 Ultra Shield instruments operating at 100 MHz. Chemical shifts (δ) of the ^{1}H and ^{13}C NMR spectra are reported in ppm with a solvent resonance as an internal standard. For ^{1}H NMR: chloroform-*d* 7.26, acetone-d_6 2.05, methanol-d_4 3.31; for ^{13}C NMR: chloroform-*d* 77.16, acetone-d_6 29.84, methanol-d_4 49.0. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, dd = doublet of a doublet, ddd = doublet of a doublet of doublet, t = triplet, dt = doublet of a triplet, q = quartet, quint = quintet, m = multiplet, br = broad, ar = aromatic. Gas chromatography-mass spectrometry experiments were performed on Agilent 7890B Gas Chromatograph (GC) System (Agilent Technologies). Electrospray ionization (ESI) mass spectrometry (MS) experiments were performed on an Agilent 6530 Accurate-Mass Q-TOF LC/MS system (Agilent Technologies). Optical rotations were measured on a DigiPol 781 M6U Automatic Polarimeter. CD spectroscopy was carried out on a Jasco J-1500 spectrophotometer (Jasco International Co.). UV spectroscopy and activity measurements were performed on Cary 300 UV/Vis spectrophotometer (Agilent Technologies).

II. Molecular cloning, bacterial expression and activity measurements

DNA preparation and transformation

T_4HNR_*his* and T_3HNR_*his* plasmids cloned into the pET-19b vector has been obtained from Prof. Michael Müller, University of Freiburg and were used as per procedure reported elsewhere.[1] Glucose dehydrogenase (GDH) gene was provided by Prof. Werner Hummel (University of Bielefeld, Germany). Transformation of plasmid DNA to competent *E. coli* BL21(DE3) cells was performed by applying a heat shock at 42 °C for 45 s.

Media and growth conditions

One clone was picked and dispersed in 5 mL of LB-media (Lennox) containing ampicillin (100 $\mu g \cdot mL^{-1}$), followed by incubation overnight (37 °C, 220 rpm).

Cultivation and expression

Overnight cultures (5 mL each) were diluted to 500 mL of LB (Lennox) media containing ampicillin (100 $\mu g \cdot mL^{-1}$) and incubated at 37 °C, 160 rpm. The protein expression was induced by adding IPTG (0.2 mM) for T_4HNR_*his*, T_3HNR_*his* and GDH during the mid-log phase (OD600nm = 0.6), followed by further incubation for 4 h at 37 °C, 160 rpm for T_4HNR_*his*, T_3HNR_*his* and GDH.

Workup and storage

The harvested *E. coli* cells were resuspended in lysis buffer (50 mM HEPES, pH = 7.5; 2.5 mL per harvested cells of 500 mL culture). The cells were disrupted by sonication (8 times 10 sec, Vibra-Cell Processors, model no. VCX500, Sonics), followed by centrifugation (30 min, 12000 *g*, 4 °C). Glycerol (20% v/v) was added and the crude enzyme preparation was frozen at –20 °C.

Activity measurements

T_4HNR, T_3HNR, GDH were assayed as described elsewhere.[1]

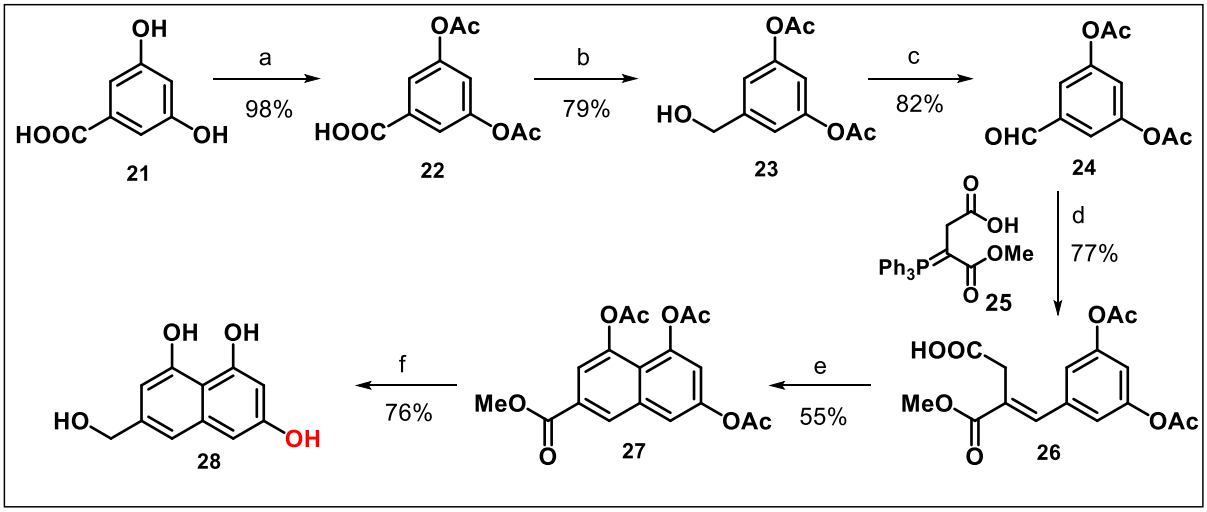
III. Sequence alignment studies

Entry	Protein Accession ID	Sequence identity (%) with T_4HNR of *M. grisea*	Sequence identity (%) with T_3HNR of *M. grisea*
1	AFO12494.1 of *D. eschscholtzii*	50	45
2	AFO12495.1 of *D. eschscholtzii*	53	82

Table S1. Sequence alignment studies of T_4HNR and T_3HNR of *M.grisea* with *Daldinia eschscholtzii* genome

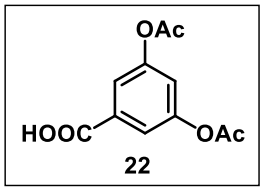
IV. Synthesis of substrates 28, 17, 18

a. Synthesis of 6-(hydroxymethyl) naphthalene-1,3,8-triol (28)



Scheme S1. Synthesis of 6-(hydroxymethyl) naphthalene-1,3,8-triol (**28**). (a) Ac_2O / Py, 70 °C, 3 h. (b) BMS, THF (dry), 0 °C, 30 min. (c) PCC, NaOAc, DCM (dry), 1 h. (d) Benzene (dry), rt, 5 d. (e) Ac_2O, AcOK, reflux, 15 min. (f) LAH, THF (dry), 0 °C, 2 h.

3,5-diacetoxybenzoic acid (22)



$C_{11}H_{10}O_6$: 238.19 g. mol^{-1}

3,5-dihydroxy benzoic acid **21** (3 g, 19.48 mmol, 1 equiv.) was dissolved in acetic anhydride (6 ml, 58.96 mmol, 3 equiv.) and pyridine (20 ml). The reaction mixture was heated at 70 ^{o}C for 3 h. After completion of the reaction, monitored by TLC, the reaction mixture was cooled to rt, extracted with ethyl acetate (2×), washed with brine and dried over anhydrous sodium sulphate. The extracted organic layer was concentrated on the rotary evaporator and purified by the column chromatography (Silica gel 60-120 mess size, Hexane: EtOAc 1:1) to afford 3,5-diacetoxybenzoic acid **22** (4.54 g, 98% yield) as a white solid.

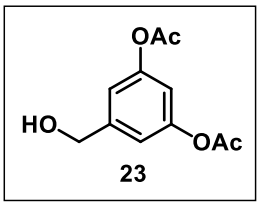
TLC (hexane/ethyl acetate, 7:3 v/v): R_f = 0.40;

^{1}H NMR: (400 MHz, $CDCl_3$) δ [ppm]: 2.32 (s, 6H, $OCOCH_3$), 7.20 (t, 1H, ^{4}J = 2.1 Hz, CH_{ar}), 7.72 (d, 2H, ^{4}J = 2.1 Hz, CH_{ar}).

^{13}C NMR: (100 MHz, $CDCl_3$) δ [ppm]: 21.2, 121.0, 121.1, 131.4, 151.2, 168.9, 169.7.

HRMS (ESI) m/z: [M + H] $^{+}$ 239.0550 (calculated); 239.0581 (found).

5-(hydroxymethyl)-1,3-phenylene diacetate (23)



23

$C_{11}H_{12}O_5$: 224.21 g. mol^{-1}

3,5-diacetoxybenzoic acid **22** (4.5 g, 18.9 mmol, 1 equiv.) was dissolved in anhydrous THF (142 ml). BMS (66 ml, 2 M in THF, 132.35 mmol, 7 equiv.) was added at 0^{o}C. The reaction mixture was stirred for 15 min at 0^{o}C. The reaction was monitored by TLC, after the consumption of acid, the reaction was quenched by adding water, and the mixture was extracted with diethyl ether (2×), washed with saturated brine solution and dried over anhydrous sodium sulphate. The extracted organic layer was concentrated on a rotary evaporator and purified by the column chromatography (Silica gel, 60-120 mess size, Hexane: EtOAc 7:3) to afford 5-(hydroxymethyl)-1,3-phenylene diacetate **23** (3.34 g, 79% yield) as a colorless liquid.

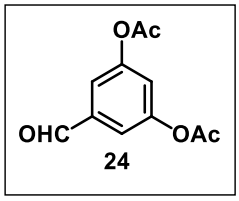
TLC (hexane/ethyl acetate, 1:1 v/v): R_f = 0.44;

^{1}H NMR: (400 MHz, $CDCl_3$) δ [ppm]: 2.28 (s, 6H, $OCOCH_3$), 4.65 (s, 2H, CH_2), 6.81 (t, ^{4}J = 1.4 Hz, 1H, CH_{ar}), 6.98 (d, ^{4}J = 1.4 Hz, 2H, CH_{ar}).

^{13}C NMR: (100 MHz, $CDCl_3$) δ [ppm]: 21.2, 64.3, 143.8, 114.4, 117.3, 151.2, 169.3.

HRMS (ESI) m/z: [M + Na]$^{+}$ 247.0577 (calculated); 247.0578 (found).

5-formyl-1,3-phenylene diacetate (24)



24

$C_{11}H_{10}O_5$: 222.20 g. mol^{-1}

To a solution of PCC (9.213 g, 42.66 mmol, 2 equiv.) and sodium acetate (1.24 g, 15.17 mmol, 0.71 equiv.) in anhydrous DCM (16 mL) was added a solution of 5-(hydroxymethyl)-1,3-phenylene diacetate **23** (4.78 g, 21.33 mmol, 1 equiv.) in anhydrous DCM (100 mL). The mixture was stirred under nitrogen for 1 h and treated with diethyl ether (70 mL). The brown mixture was filtered through sintered funnel over celite. The filtrate was concentrated on a rotary evaporator and the residue was subjected to column chromatography (Silica gel, 60-120 mess size, Hexane: EtOAc 85:15) to afford compound 5-formyl-1,3-phenylene diacetate **24** (3.64 g, 77% yield) as a pale-yellow liquid.

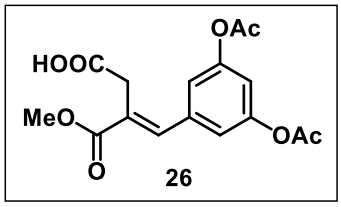
TLC (hexane/ethyl acetate, 1:1 v/v): R_f = 0.72;

^{1}H NMR: (400 MHz, $CDCl_3$) δ [ppm]: 2.32 (s, 6H, $OCOCH_3$), 7.21 (t, ^{4}J = 1.98 Hz, 1H, CH_{ar}), 7.52 (d, ^{4}J = 1.98 Hz, 2H, CH_{ar}), 9.96 (s, 1H, CHO).

^{13}C NMR: (100 MHz, $CDCl_3$) δ [ppm]: 21.2, 120.1, 121.5, 138.2, 151.7, 168.8, 190.2.

HRMS (ESI) m/z: [M + H]$^{+}$ 223.0601 (calculated); 223.0598 (found).

(*E*)-4-(3,5-diacetoxyphenyl)-3-(methoxycarbonyl) but-3-enoic acid (26)



$C_{16}H_{16}O_8$: 336.30 g. mol^{-1}

Compound (**26**) was synthesised using the procedure as described in the literature[2]. The 5-formyl-1,3-phenylene diacetate **24** (1 g, 4.50 mmol, 1 equiv.) and ylide **25** (1.94 g, 4.95 mmol, 1.1 equiv.) (**25** was synthesised as reported in the literature[3]) were dissolved in anhydrous benzene (15 mL) and stirred at rt for 6d. After consumption of starting material, the solvent was removed under reduced pressure. Then the residue was subjected to column chromatography (Silica gel, 60-120 mess size, Hexane: EtOAc 1:1) to afford compound **26** (1.15 g, 76% yield) as a viscous pale-yellow liquid.

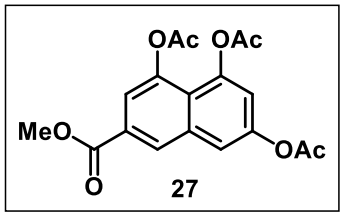
TLC (hexane/ethyl acetate, 1:1 v/v): R_f = 0.24;

^{1}H NMR: (400 MHz, $CDCl_3$) δ [ppm]: 2.29 (s, 6H, $OCOCH_3$), 3.55 (s, 2H, CH_2), 3.83 (s, 3H, CH_3), 6.93 (t, ^4J = 1.7 Hz, 1H, CH_{ar}), 6.98 (d, ^4J = 1.3 Hz, 2H, CH_{ar}), 7.83 (d, 1H, CH).

^{13}C NMR: (100 MHz, $CDCl_3$) δ [ppm]: 21.2, 33.6, 52.7, 116.1, 119.5, 127.2, 136.6, 140.6, 151.2, 167.5, 169.0, 176.3.

HRMS (ESI) m/z: $[M + H]^+$ 337.0918 (calculated); 337.0917 (found).

6-(methoxycarbonyl) naphthalene-1,3,8-triyl triacetate (27)



$C_{18}H_{16}O_8$: 360.32 g. mol^{-1}

Compound (**27**) was synthesised using the procedure as described in the literature[3].The (3,5-diacetoxyphenyl)-3-(methoxycarbonyl) but-3-enoic acid **26** (1.19g, 3.54 mmol, 1 equiv.) and sodium acetate (348 mg, 4.25 mmol, 1.2 equiv.) were refluxed in acetic anhydride (7 mL) for 15 min. Then the solution was poured into water and added ethyl acetate to. The mixture was

extracted with ethyl acetate, dried over anhydrous sodium sulphate and the solvent was evaporated on a rotary evaporator. The crude product was purified by column chromatography (Silica gel, 60-120 mess size, Hexane: EtOAc 1:1) to afford compound **27** (700 mg, 55% yield) as a pale-yellow solid.

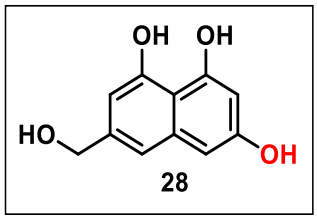
TLC (hexane/ethyl acetate, 1:1 v/v): R_f = 0.60;

^{1}H NMR: (400 MHz, $CDCl_3$) δ [ppm]: 2.34 (s, 3H, $OCOCH_3$), 2.39 (s, 3H, $OCOCH_3$), 2.40 (s, 3H, $OCOCH_3$), 3.96 (s, 3H, OCH_3), 7.12 (d, ^{4}J = 1.8 Hz, 1H, CH_{ar}), 7.67 (d, ^{4}J = 1.7 Hz, 1H, CH_{ar}), 7.69 (s, 1H, CH_{ar}), 8.47 (s, 1H, CH_{ar}).

^{13}C NMR: (100 MHz, $CDCl_3$) δ [ppm]: 21.2, 52.7, 118.7, 118.9, 119.9, 121.5, 128.8, 129.3, 135.8, 145.6, 145.9, 148.6, 165.8, 168.8, 169.0, 169.3.

HRMS (ESI) m/z: [M + H] $^{+}$ 361.0918 (calculated); 361.0893 (found).

6-(hydroxymethyl) naphthalene-1,3,8-triol (28)



28

$C_{11}H_{10}O_4$: 206.20 g. mol^{-1}

The compound 6-(methoxycarbonyl) naphthalene-1,3,8-triyl triacetate **27** (700 mg, 1.94 mmol, 1 equiv.) was dissolved in anhydrous THF (40 mL) and added dropwise to the suspension of lithium aluminium hydride (737.9 mg, 19.4 mmol, 10 equiv.) in dry ether (10 mL). Then stirred the solution at rt for 2 h. The reaction was monitored by checking TLC. After consumption of starting material, the reaction mixture was quenched by adding saturated NH_4Cl. Then the mixture was extracted with ether, dried over anhydrous sodium sulphate and concentrated on a rotary evaporator. The crude product was subjected to column chromatography (Silica gel, 60-120 mess size, CH_2Cl_2: MeOH 9:1) to afford compound **28** (308 mg, 76% yield) as a white solid.

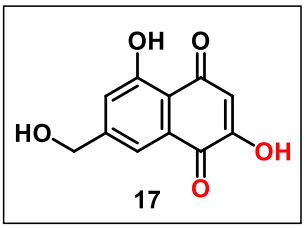
TLC (chloroform/methanol, 9:1 v/v): R_f = 0.40;

^{1}H NMR: (400 MHz, acetone-d_6) δ [ppm]: 4.61 (s, 2H, CH_2), 6.41 (d, ^{4}J = 1.5 Hz, 1H, CH_{ar}), 6.59 (s, 1H, CH_{ar}), 6.63 (d, ^{4}J = 1.5 Hz, 1H, CH_{ar}), 7.06 (s, 1H, CH_{ar}), 8.51 (s, 1H, OH), 9.97 (s, 1H, OH), 10.03 (s, 1H, OH).

^{13}C NMR: (100 MHz, acetone-d_6) δ [ppm]: 64.7, 101.5, 102.3, 105.7, 109.9, 115.9, 139.1, 142.5, 155.0, 156.5, 157.3.

HRMS (ESI) m/z: [M + H] $^{+}$ 207.0652 (calculated); 207.0651 (found).

2,5-dihydroxy-7-(hydroxymethyl)naphthalene-1,4-dione (17)



17

$C_{11}H_8O_5$: 220.18 g. mol^{-1}

K_2CO_3 (268 mg, 1.94 mmol, 4.0 equiv.) was added to a solution of compound **28** (100 mg, 0.49 mmol, 1.0 equiv.) in DMF (2.9 ml). The resultant mixture was stirred at 25 °C for 2 h, open to the air. Upon completion, the reaction contents were slowly acidified with concentrated HCl until a pH of 2 was reached. The resultant mixture was poured into water (5 mL) and extracted with Et_2O (2×). The combined organic layers were washed with water dried (Na_2SO_4) and concentrated on a rotary evaporator. The resultant crude solid was purified by column chromatography (Silica gel, 60-120 mess size, ether/methanol, 9:1 v/v) to afford **17** (89 mg, 84% yield) as an orange solid.

TLC (chloroform/methanol, 85:15 v/v): R_f = 0.2;

^{1}H NMR: (400 MHz, acetone-d_6) δ [ppm]: 4.74 (s, 2H, CH_2), 6.20 (s, 1H, CH_{ar}), 7.29 (s, 1H, CH_{ar}), 7.62 (s, 1H, CH_{ar}), 12.40 (s, 1H, OH).

^{13}C NMR: (100 MHz, acetone-d_6) δ [ppm]: 63.6, 111.2, 113.9, 117.6, 122.6, 131.4, 152.3, 160.3, 162.1, 181.8, 192.4.

HRMS (ESI) m/z: [M + H]$^{+}$ 221.0444 (calculated); 221.0441 (found).

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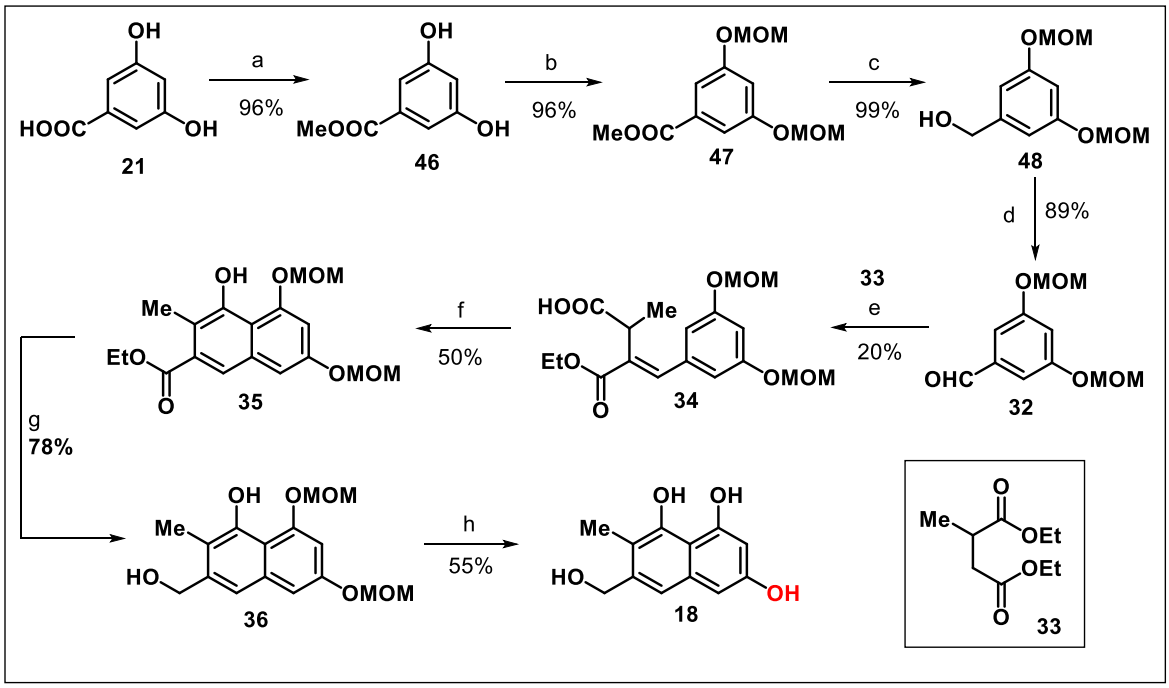
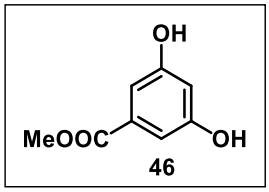
b. Synthesis of 6-(hydroxymethyl)-7-methylnaphthalene-1,3,8-triol (18)



Scheme S2. Synthesis of 6-(hydroxymethyl)-7-methylnaphthalene-1,3,8-triol (**18**). (a) MeOH, H_2SO_4 (c), reflux, 3 h. (b) MOM-Cl, DIPEA, DCM (dry), 0 ^oC. 12 h. (c) LAH, THF (dry), 0 ^oC, 2 h. (d) PCC, NaOAc, DCM (dry), 1 h. (e) NaH, Toluene (dry), cat. EtOH, rt, 1 h. (f) Ac_2O, AcOK, reflux, 15 min. (g) LAH, THF (dry), 0 ^oC, 1 h. (h) MeOH, HCl (c), 60 ^oC, 30 min.

Methyl 3,5-dihydroxybenzoate (46)



$C_8H_8O_4$: 168.15 g. mol^{-1}

3,5-dihydroxybenzoic acid **21** (3 g, 19.48 mmol, 1 equiv.) was dissolved in methanol (15mL) followed by the addition of conc. H_2SO_4 (1 mL). Then, the reaction mixture was heated at 70^oC for 3h. The reaction was monitored by TLC. After completion, the reaction mixture was cooled to rt, extracted with ethyl acetate, washed with water and dried over anhydrous sodium sulphate. Then extracted organic layer was concentrated on a rotary evaporator followed by column chromatography (Silica gel 60-120 mess size, Hexane: EtOAc 1:1) to afford methyl 3,5-dihydroxybenzoate **46** (3.14 g, 96 % yield) as a colourless solid.

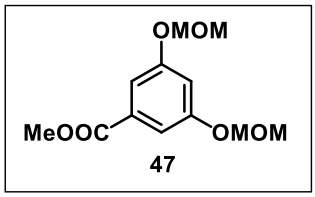
TLC (hexane/ethyl acetate, 7:3 v/v): R_f = 0.56;

^{1}H NMR: (400 MHz, acetone-d_6) δ [ppm]: 3.82 (s, 3H, $COOCH_3$), 6.58 (t, ^4J = 1.9 Hz, 1H, CH_{ar}), 7.00 (d, ^4J = 1.9 Hz, 2H, CH_{ar}), 8.56 (s, 2H, OH).

^{13}C NMR: (100 MHz, acetone-d_6) δ [ppm]: 52.2, 107.9, 108.6, 133.0, 159.4, 167.1.

HRMS (ESI) m/z: [M + H]$^+$ 169.0495 (calculated); 169.0491 (found).

Methyl 3,5-bis(methoxymethoxy)benzoate (47)[4]



47

$C_{12}H_{16}O_6$: 256.25 g. mol^{-1}

To a solution of methyl 3,5-dihydroxybenzoate **46** (3.15 g, 18.75 mmol, 1 equiv.) in anhydrous CH_2Cl_2 (60 mL) was added diisopropylethylamine (12.54 mL, 75 mmol, 4 equiv.) followed by MOMCl (15.9 mL, 37.5 mmol, 2 equiv.) dropwise at 0°C. The mixture was warmed to rt and stirred for 12 h. The mixture was then quenched with water and diluted with CH_2Cl_2 (50 mL). The organic layer was separated, washed with saturated $NaHCO_3$ solution, and extracted with CH_2Cl_2. The combined organic layer was washed with brine, dried over anhydrous sodium sulphate, and concentrated under reduced pressure. The resulting residue was subjected to silica gel column chromatography (Silica gel, 60-120 mess size, Hexane: EtOAc 7:3) to afford compound Methyl 3,5-bis(methoxymethoxy)benzoate **47** (4.60 g, 96 % yield) as a colourless oil**.**

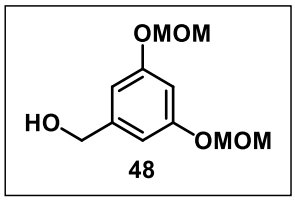
TLC (hexane/ethyl acetate, 7:3 v/v): R_f = 0.6;

^{1}H NMR: (400 MHz, $CDCl_3$) δ [ppm]: 3.46 (s, 6H, OCH_3), 3.88 (s, 3H, $COOCH_3$), 5.17 (s, 4H, OCH_2O), 6.90 (t, ^4J = 1.7 Hz, 1H, CH_{ar}), 7.35 (d, ^4J = 1.7 Hz, 2H, CH_{ar}).

^{13}C NMR: (100 MHz, $CDCl_3$) δ [ppm]: 52.3, 94.5, 109.8, 110.7, 132.3, 158.2, 166.6.

HRMS (ESI) m/z: [M + H]$^+$ 257.1020 (calculated); 257.1021 (found).

(3,5-bis(methoxymethoxy)phenyl) methanol (48) [4]



$C_{11}H_{16}O_5$: 228.24 g. mol^{-1}

Methyl 3,5-bis(methoxymethoxy)benzoate **47** (4.5 g, 17.57 mmol, 1 equiv.) was dissolved in anhydrous THF (50 ml) and added dropwise to the suspension of lithium aluminium hydride (800 mg, 21.08 mmol, 1.2 equiv.) in dry THF (10 mL). Then stirred the solution at rt for 2 h. The reaction was monitored by checking TLC. After consumption of starting material, the reaction mixture was quenched by adding dilute HCl. Then the mixture was extracted with ethyl acetate, dried over anhydrous sodium sulphate. The extracted organic layer was concentrated on a rotary evaporator and purified by the column chromatography (Silica gel, 60-120 mess size, Hexane: EtOAc 7:3) to afford (3,5-bis(methoxymethoxy)phenyl) methanol **48** (3.99 g, 99% yield) as a colourless liquid.

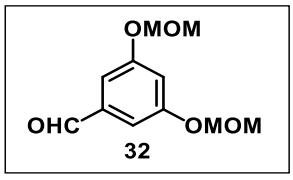
TLC (hexane/ethyl acetate, 7:3 v/v): R_f = 0.2;

^{1}H NMR: (400 MHz, $CDCl_3$) δ [ppm]: 3.45 (s, 6H, OCH_3), 4.59 (s, 2H, CH_2), 5.13 (s, 4H, OCH_2O), 6.62 (t, ^4J = 1.5 Hz, 1H, CH_{ar}), 6.68 (d, ^4J = 1.5 Hz, 2H, CH_{ar}).

^{13}C NMR: (100 MHz, $CDCl_3$) δ [ppm]: 56.1, 65.1, 94.5, 104.1, 108.0, 143.7, 158.5.

HRMS (ESI) m/z: $[M + H]^+$ 229.1071 (calculated); 229.1071 (found).

3,5-bis(methoxymethoxy)benzaldehyde (32) [4]



$C_{11}H_{14}O_5$: 226.22 g. mol^{-1}

To a solution of PCC (8.92 g, 41.40 mmol, 2 equiv.) and sodium acetate (1.21 g, 14.69 mmol, 0.71 equiv.) in anhydrous DCM (67 mL) was added a solution of (3,5-

bis(methoxymethoxy)phenyl) methanol **48** (4.72 g, 20.70 mmol, 1 equiv.) in anhydrous DCM (5 mL). The mixture was stirred under nitrogen for 1 hr and treated with diethyl ether (50 mL). The brown mixture was filtered through sintered funnel over celite. The filtrate was concentrated on a rotary evaporator and the residue was subjected to column chromatography (Silica gel, 60-120 mess size, Hexane: EtOAc 7:3) to afford compound 3,5-bis(methoxymethoxy)benzaldehyde **32** (4.16 g, 89% yield) as a pale-yellow oil.

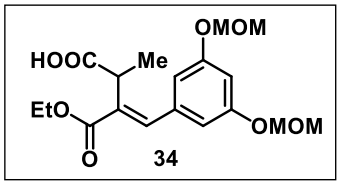
TLC (hexane/ethyl acetate, 7:3 v/v): R_f = 0.56;

^{1}H NMR: (400 MHz, $CDCl_3$) δ [ppm]: 3.47 (s, 6H, OCH_3), 5.20 (s, 4H, OCH_2O), 6.96 (t, ^4J = 2.0 Hz, 1H, CH_{ar}), 7.20 (d, ^4J = 2.0 Hz, 2H, CH_{ar}), 9.89 (s, 1H, CHO).

^{13}C NMR: (100 MHz, $CDCl_3$) δ [ppm]: 56.3, 94.6, 110.6, 111.3, 138.6, 158.9, 191.1.

HRMS (ESI) m/z: [M + H]$^+$ 227.0914 (calculated); 227.0907 (found).

4-(3,5-bis(methoxymethoxy)phenyl)-3-(ethoxycarbonyl)-2-methylbut-3-enoic acid (34)



34

$C_{18}H_{24}O_8$: 368.38 g. mol^{-1}

To a well-stirred mixture of sodium hydride (126 mg, 5.26 mol, 2.38 equiv., prepared from 221 mg of a 60% mineral oil dispersion which had been previously washed twice with pentane and once with toluene) in anhydrous toluene (3 mL) under nitrogen and at rt was added a catalytic amount of absolute ethanol (5 µL) followed by dropwise addition of a solution of diethyl succinate **33** (1.04 g, 5.53 mmol, 2.5 equiv.) and 3,5-bis(methoxymethoxy)benzaldehyde **32** (500 mg, 2.21 mmol, 1 equiv.). The rate of addition was sufficient to maintain a steady evolution of hydrogen and a reaction temperature not exceeding 56 ^{o}C (**33** was made following the reported procedure[5]). The mixture was stirred for 1h at rt, and then conc. HCl followed by water was added. The mixture was extracted with ethyl acetate, dried over anhydrous sodium sulphate and the solvent was evaporated on a rotary evaporator. The crude product was purified by column chromatography (Silica gel, 60-120 mess size, Hexane: EtOAc 1:1) to afford compound **34** (163 mg, 20% yield) as a pale-yellow liquid.

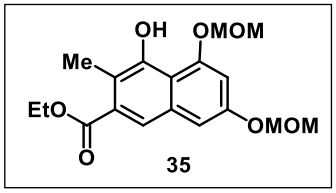
TLC (hexane/ethyl acetate, 1:1 v/v): R_f = 0.24;

^{1}H NMR: (400 MHz, $CDCl_3$) δ [ppm]: 1.31 (t, *J* = 7.2 Hz, 3H, CH_3), 1.41 (d, *J* = 7.0 Hz, 3H, CH_3), 3.47 (s, 6H, OCH_3), 3.90 (q, *J* = 7.0 Hz, 1H, CH), 4.21–4.29 (m, 2H, CH_2), 5.15 (s, 4H, OCH_2O), 6.70 (s, 2H, CH_{ar}), 6.72 (s, 1H, CH_{ar}), 7.73 (s, 1H, CH).

^{13}C NMR: (100 MHz, $CDCl_3$) δ [ppm]: 14.2, 15.9, 38.4, 56.2, 61.3, 94.7, 105.3, 110.2, 133.1, 137.1, 140.6, 158.6, 166.6, 179.1.

HRMS (ESI) m/z: $[M + H]^+$ 369.1544 (calculated); 369.1544 (found).

Ethyl 4-acetoxy-5,7-bis(methoxymethoxy)-3-methyl-2-naphthoate (35)



35

$C_{18}H_{22}O_7$: 350.37 g. mol^{-1}

34 (517 mg, 1.40 mmol, 1 equiv.) and sodium acetate (138 mg, 1.68 mmol, 1.2 equiv.) were refluxed in acetic anhydride (2.65 mL) for 15 min. Then the solution was poured into water and added ethyl acetate to. The mixture was extracted with ethyl acetate, dried over anhydrous sodium sulphate and the solvent was evaporated on a rotary evaporator. The crude product was purified by column chromatography (Silica gel, 60-120 mess size, Hexane: EtOAc 1:1) to afford compound **35** (245 mg, 50% yield) as a pale-yellow solid.

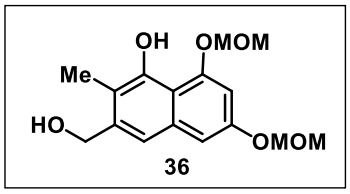
TLC (hexane/ethyl acetate, 8:2 v/v): R_f = 0.48;

^{1}H NMR: (400 MHz, $CDCl_3$) δ [ppm]: 1.42 (t, *J* = 7.1 Hz, 3H, CH_3), 2.49 (s, 3H, CH_3), 3.51 (s, 3H, OCH_3), 3.58 (s, 3H, OCH_3), 4.39 (q, *J* = 7.1 Hz, 2H, CH_2), 5.25 (s, 2H, OCH_2O), 5.43 (s, 2H, OCH_2O), 6.88 (s, 1H, CH_{ar}), 7.05 (s, 1H, CH_{ar}), 7.72 (s, 1H, CH_{ar}), 9.49 (s, 1H, OH).

^{13}C NMR: (100 MHz, $CDCl_3$) δ [ppm]: 12.4, 14.5, 56.4, 57.1, 61.1, 94.7, 96.1, 103.5, 105.2, 112.7, 117.0, 120.6, 131.9, 134.3, 152.0, 154.0, 154.8, 168.4.

HRMS (ESI) m/z: $[M + H]^+$ 351.1438 (calculated); 351.1439 (found).

3-(hydroxymethyl)-6,8-bis(methoxymethoxy)- 2-methylnaphthalen-1-ol (36)



$C_{16}H_{20}O_6$: 308.33 g. mol^{-1}

Compound **35** (232 mg, 0.66 mmol, 1 equiv.) was dissolved in anhydrous THF (10 mL) and added dropwise to the suspension of lithium aluminium hydride (50 mg, 1.32 mmol, 2 equiv.) in anhydrous THF (3 mL). Then stirred the solution at rt for 2 h. The reaction was monitored by checking TLC. After consumption of starting material, the reaction mixture was quenched by adding saturated NH_4Cl. Then the mixture was extracted with ethyl acetate, dried over anhydrous sodium sulphate and concentrated on a rotary evaporator. The crude product was subjected to column chromatography (Silica gel, 60-120 mess size, hexane/ethyl acetate, 1:1) to afford compound **36** (159 mg, 78% yield) as a white solid.

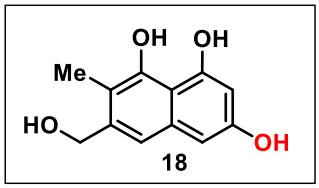
TLC (hexane/ethyl acetate, 1:1 v/v): R_f = 0.68;

^{1}H NMR: (400 MHz, $CDCl_3$) δ [ppm]: 2.29 (s, 3H, CH_3), 3.51 (s, 3H, OCH_3), 3.57 (s, 3H, OCH_3), 4.77 (s, 2H, CH_2), 5.23 (s, 2H, OCH_2O), 5.41 (s, 2H, OCH_2O), 6.79 (d, J = 2 Hz, 1H, CH_{ar}), 6.98 (d, J = 2 Hz, 1H, CH_{ar}), 7.23 (s, 1H, CH_{ar}), 9.40 (s, 1H, OH).

^{13}C NMR: (100 MHz, $CDCl_3$) δ [ppm]: 10.5, 56.3, 57.0, 64.2, 94.7, 95.9, 101.6, 104.6, 110.9, 115.6, 116.5, 135.1, 140.2, 151.3, 154.1, 154.5.

HRMS (ESI) m/z: [M + H] $^+$ 309.1333 (calculated); 309.1336 (found).

6-(hydroxymethyl)-7-methylnaphthalene-1,3,8-triol (18)



$C_{12}H_{12}O_4$: 220.22 g. mol^{-1}

To the solution of compound **36** (150 mg, 0.48 mmol) in methanol (5 ml), 2M HCl (1 ml) was added and heated at 60 ^{o}C for 30 min. After completion, the reaction was cooled to rt and water was added to it. Then the mixture was extracted with ethyl acetate, dried over anhydrous sodium sulphate and concentrated on a rotary evaporator. The crude product was subjected to column chromatography (Silica gel, 60-120 mess size, methanol/chloroform, 1:9) to afford compound **18** (59 mg, 55% yield) as a white solid.

TLC (hexane/ethyl acetate, 1:1 v/v): R_f = 0.20;

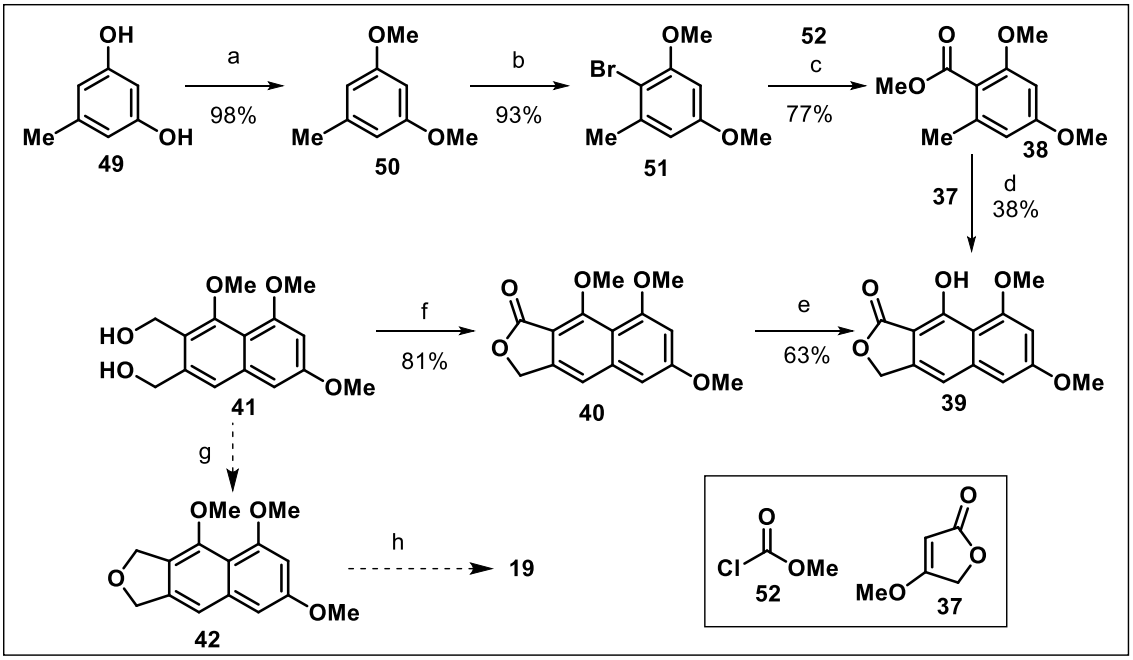
^{1}H NMR: (400 MHz, acetone-*d*$_6$) δ [ppm]: 2.09 (s, 3H, CH_3), 4.50 (s, 2H, CH_2), 5.05 (s, 1H, OH), 6.34 (s, 1H, CH_{ar}), 6.49 (s, 1H, CH_{ar}), 7.03 (s, 1H, CH_{ar}), 9.39 (s, 1H, OH), 10.00 (s, 1H, OH), 12.44 (s, 1H, OH),.

^{13}C NMR: (100 MHz, acetone-*d*$_6$) δ [ppm]: 9.9, 61.8, 100.4, 101.2, 108.3, 111.8, 114.5, 135.3, 140.7, 151.0, 153.9, 155.0.

HRMS (ESI) m/z: [M + H] $^{+}$ 221.0808 (calculated); 221.0808 (found).

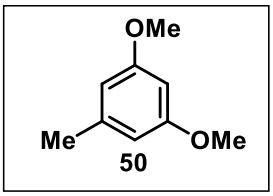
V. Attempted synthesis of substrates 19 and 20

a. Attempted Synthesis of 1,3-dihydronaphtho[2,3-c] furan-4,5,7-triol (19) from orcinol (49)



Scheme S3. Synthesis of 1,3-dihydronaphtho[2,3-c] furan-4,5,7-triol **19**. (a) DMS, K_2CO_3, acetone (dry) reflux, 12 h. (b) NBS, $CHCl_3$, reflux, 3 h. (c) nBuLi, THF (dry), –78 ^oC, 3 h. (d) LDA, THF (dry), –78 ^oC, 2.5 h. (e) DMS, K_2CO_3, acetone (dry) reflux, 12 h. (f) DIBAL-H, toluene (dry), –40 ^oC, 3 h. (g) PBr_3, DCM (dry), 0 ^oC, 5 min. (h) BBr_3, DCM (dry), –78 ^oC

1,3-dimethoxy-5-methylbenzene (50)[6]



$C_9H_{12}O_2$: 152.19 g. mol^{-1}

A mixture of orcinol **49** (1 g, 8.05 mmol, 1 equiv.), potassium carbonate (3.34 g, 24.16 mmol, 3 equiv.), and dimethyl sulphate (1.67 mL, 17.71 mmol, 2.2 equiv.) in acetone was stirred under reflux for 10 h. Then the acetone was removed under reduced pressure and added water to it. The product was extracted with ethyl acetate, dried over anhydrous sodium sulphate, solvent was evaporated on rotary evaporator. The Crude product was subjected to column

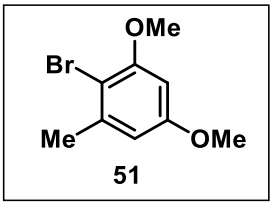
chromatography (Silica gel, 60-120 mess size, Hexane: EtOAc, 9:1) to afford compound **50** (1.20 g, 98% yield) as a colourless liquid.

TLC (hexane/ethyl acetate, 7:3 v/v): R_f = 0.8;

^{1}H NMR: (400 MHz, $CDCl_3$) δ [ppm]: 2.31 (s, 3H, CH_3), 3.78 (s, 6H, OCH_3), 6.29 (t, ^4J = 2.18 Hz, 1H, CH_{ar}), 6.34 (d, ^4J = 2.18 Hz, 2H, CH_{ar}).

^{13}C NMR: (100 MHz, $CDCl_3$) δ [ppm]: 22.0, 55.4, 97.7, 107.2, 140.4, 160.8.

2-bromo-1,5-dimethoxy-3-methylbenzene (51)[6]



51

$C_9H_{11}BrO_2$: 231.08 g. mol^{-1}

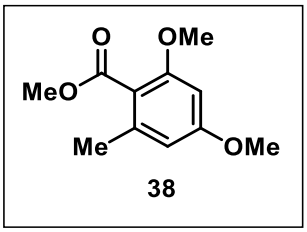
Compound **50** (1.09 g, 7.07 mmol, 1 equiv.) was dissolved in $CHCl_3$ (10 ml), NBS (1.385 g, 7.78 mmol, 1.1 equiv.) was added to it, and reflux for 3 h. Reaction was monitored (checking TLC) and after consumption of SM reaction mixture was quenched with water. The product was extracted with DCM, dried over anhydrous sodium sulphate, solvent was evaporated under reduced pressure. The crude product was purified by column chromatography (Silica gel, 60-120 mess size, Hexane: EtOAc, 95:5) to afford compound **51** (1.54 g, 93% yield) as a pale-yellow solid.

TLC (hexane/ethyl acetate, 9:1 v/v): R_f = 0.68;

^{1}H NMR: (400 MHz, $CDCl_3$) δ [ppm]: 2.39 (s, 3H, CH_3), 3.79 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 6.35 (d, ^4J = 2.62 Hz, 1H, CH_{ar}), 6.43 (d, ^4J = 2.52 Hz, 1H, CH_{ar}).

^{13}C NMR: (100 MHz, $CDCl_3$) δ [ppm]: 23.7, 55.6, 56.4, 97.4, 105.2, 107.3, 139.9, 156.7, 159.4.

Methyl 2,4-dimethoxy-6-methylbenzoate (38)[6]



38

$C_{11}H_{14}O_4$: 210.23 g. mol^{-1}

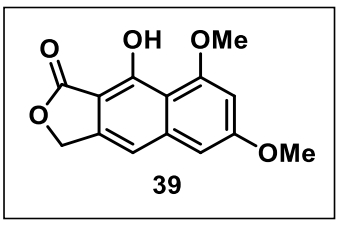
To a stirring solution of **51** (1.389 g, 6.03 mmol, 1 equiv.) in THF (14 ml) at −78 °C was added n-BuLi (2.9 mL, 2M in THF, 7.24 mmol, 1.2 equiv.) dropwise. After 1 h stirring methyl chloroformate **52** (1.717 g, 18.17 mmol, 3.0 equiv.) was added dropwise. Then the mixture was continued for further 1 hr at the same temperature. The mixture was warmed to rt and keep stirring for another 1 h. After that reaction was quenched by adding saturated ammonium chloride, extracted with ethyl acetate, dried over anhydrous sodium sulphate, and concentrated under vacuum. The Crude product was subjected to column chromatography (Silica gel, 60-120 mess size, Hexane: EtOAc, 8:2) to afford compound **38** (0.969 g, 77% yield) as a colourless liquid.

TLC (hexane/ethyl acetate, 7:3 v/v): R_f = 0.6;

^{1}H NMR (400 MHz, $CDCl_3$) δ [ppm]: 2.26 (s, 3H, CH_3), 3.76 (s, 6H, OCH_3), 3.85 (s, 3H, OCH_3), 6.29 (s, 2H, CH_{ar}).

^{13}C NMR (100 MHz, $CDCl_3$) δ [ppm]: 19.9, 52.0, 55.3, 55.9, 96.1, 106.7, 116.4, 138.2, 158.2, 161.4, 168.7.

9-hydroxy-6,8-dimethoxynaphtho[2,3-c] furan-1(3H)-one (39)



39

$C_{14}H_{12}O_5$: 260.25 g. mol^{-1}

To a stirred solution of LDA (7.14 ml, 7.14 mmol, 1 M in THF, 3 equiv.) in anhydrous THF (10 mL) at −78 °C was injected a solution of Methyl 2,4-dimethoxy-6-methylbenzoate **38** (500 mg, 2.38 mmol, 1 equiv.) in anhydrous THF (5 ml) and the solution was stirred for 1 h. A

solution of 3-methoxycyclopent-2-en-1-one **37** (271 mg, 2.38 mmol, 1 equiv.) in anhydrous THF (5 mL) was added dropwise to the resultant orange red solution and the reaction mixture was stirred for 1 h at the same temperature. After that the mixture was warmed at rt and stirred for another 1 h. The dil HCl was added to the mixture (pH-2). The product was extracted with DCM, washed with brine, dried over anhydrous sodium sulphate and concentrated under vacuum. The residue was purified by column chromatography (Silica gel, 60-120 mess size, dichloromethane/acetone, 98:2) to afford compound **39** (235 mg, 38% yield) as a solid.

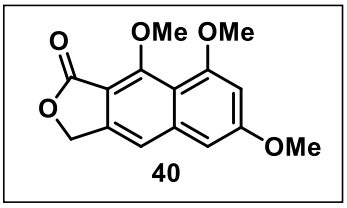
TLC (dichloromethane/acetone, 7:3 v/v): R_f = 0.52;

^{1}H NMR (400 MHz, $CDCl_3$) δ [ppm]: 3.92 (s, 3H, CH_3), 4.08 (s, 3H, OCH_3), 5.29 (s, 2H, CH_2), 6.51 (s, 1H, CH_{ar}), 6.71 (s, 1H, CH_{ar}), 7.09 (s, 1H, CH_{ar}), 10.18 (s, 1H, OH).

^{13}C NMR (400 MHz, $CDCl_3$) δ [ppm]: 55.7, 56.7, 68.7, 98.5, 99.9, 104.9, 110.0, 110.3, 141.9, 143.9, 156.9, 159.4, 160.7, 169.9.

HRMS (ESI) m/z: [M + H] + 261.0757 (calculated); 261.0716 (found).

6,8,9-trimethoxynaphtho[2,3-c] furan-1(3H)-one (40)



40

$C_{15}H_{14}O_5$: 274.27 g. mol^{-1}

A mixture of **39** (314 mg, 1.20 mmol, 1 equiv.), potassium carbonate (500 mg, 3.62 mmol, 3 equiv.), and dimethyl sulphate (0.25 ml, 2.66 mmol, 2.2 equiv.) in acetone was stirred under reflux for10 h. Then the acetone was removed under reduced pressure and added water to it. The product was extracted with ethyl acetate, dried over anhydrous sodium sulphate, solvent was evaporated on rotary evaporator. The Crude product was subjected to column chromatography (Silica gel, 60-120 mess size, Hexane: EtOAc, 9:1) to afford compound **40** (208 mg, 63% yield) as a white solid.

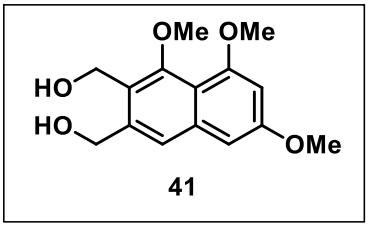
TLC (hexane/ethyl acetate, 1:1 v/v): R_f = 0.52;

^{1}H NMR (400 MHz, $CDCl_3$) δ [ppm]: 3.93 (s, 3H, CH_3), 3.98 (s, 3H, CH_3), 4.10 (s, 3H, CH_3), 5.29 (s, 2H, CH_2), 6.53 (d, ^{4}J = 1.65 Hz, 1H, CH_{ar}), 6.71 (d, ^{4}J = 1.72 Hz, 1H, CH_{ar}), 7.36 (s, 1H, CH_{ar}).

^{13}C NMR (100 MHz, $CDCl_3$) δ [ppm]: 55.6, 56.4, 63.7, 68.3, 98.8, 99.5, 112.2, 114.9, 116.5, 142.5, 143.1, 159.6, 159.8, 160.9, 168.8.

HRMS (ESI) m/z: [M + H] + 275.0914 (calculated); 275.0908 (found).

(1,6,8-trimethoxynaphthalene-2,3-diyl) dimethanol (41)



41

$C_{15}H_{18}O_5$: 278.30 g. mol^{-1}

A solution of **40** (150 mg, 0.547 mmol, 1 equiv.) in anhydrous DCM (5 ml) was cooled to –40 ^{o}C. After 15 min DIBAL-H (2.80 ml, 1M in toluene, 4.38 mmol, 8 equiv.) was added dropwise and reaction mixture was stirred for 1h at same temperature. Then it warm to rt followed by addition of water. The product was extracted with ethyl acetate, dried over anhydrous sodium sulphate, solvent was evaporated on rotary evaporator. The Crude product was subjected to column chromatography (Silica gel, 60-120 mess size, Hexane: EtOAc, 9:1) to afford compound **41** (123 mg, 81% yield) as a solid.

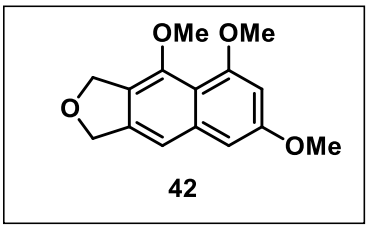
TLC (hexane/ethyl acetate, 1:1 v/v): R_f = 0.36;

^{1}H NMR (400 MHz, $CDCl_3$) δ [ppm]: 3.78 (s, 3H, CH_3), 3.86 (s, 3H, CH_3), 3.94 (s, 3H, CH_3), 4.73 (s, 3H, CH_3), 4.85 (s, 2H, CH_2), 6.49 (d, ^{4}J = 1.44 Hz, 1H, CH_{ar}), 6.61 (d, ^{4}J = 1.44 Hz, 1H, CH_{ar}), 7.33 (s, 1H, CH_{ar}).

^{13}C NMR (100 MHz, $CDCl_3$) δ [ppm]: 55.4, 56.1, 56.6, 63.7, 64.7, 99.1, 99.3, 115.2, 124.0, 127.2, 137.6, 139.4, 155.4, 157.2, 158.5.

HRMS (ESI) m/z: [M + Na] + 301.1046 (calculated); 301.1045 (found).

4,5,7-trimethoxy-1,3-dihydronaphtho[2,3-c]furan (42)



Entry	Reagents	Observation in NMR
1	MnO_2, Et_3SiH, TFA, DCM, (-5^oC to rt)	No desired product
2	DMC, NaOMe, CH_3CN	No desired product
3	BuLi, TsCl, BuLi, heat	No desired product
4	Ag_2O, TsCl, KI, rt	No desired product
5	Et_3N, MsCl, DCM, rt	No desired product
6	NaH, $PO(OMe)_3$, rt, 24 h	No desired product
7	PBr_3, DCM, 0^oC, 5 min	Desired product

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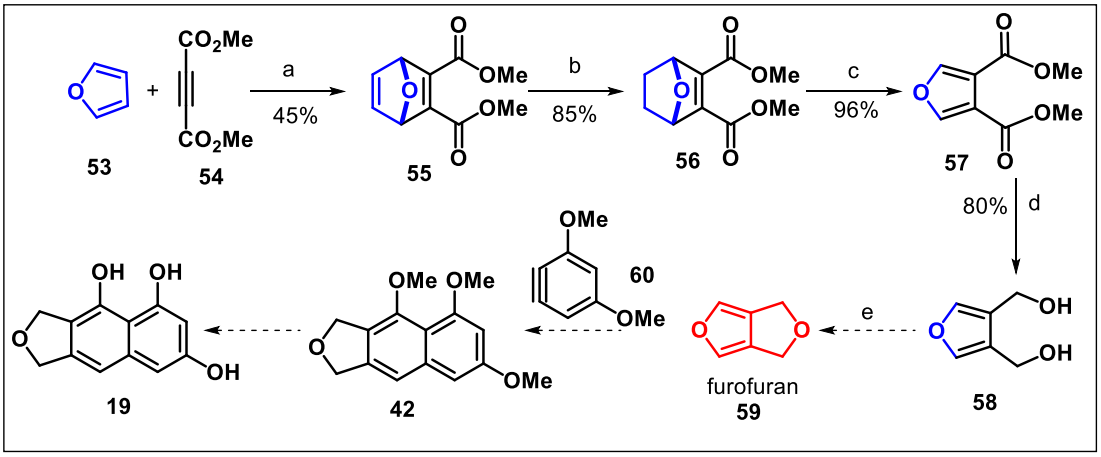
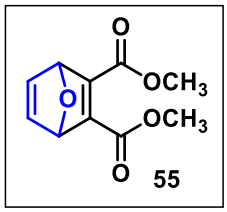
b. Attempted Synthesis of 1,3-dihydronaphtho[2,3-c] furan-4,5,7-triol (19) starting from furan (53)



Scheme S4. Synthesis of 1,3-dihydronaphtho[2,3-c] furan-4,5,7-triol **19**. (a) 100 ^oC, 18 h. (b) 10% Pd / C, H_2 6 h. (c) 175 ^oC, 1 h. (d) $LiAlH_4$, Ether (dry), 0 ^oC - rt, 1 h. (e) MnO_2, Triethylsilane, TFA, DCM (-5 ^oC to rt).

Dimethyl 7- oxabicyclo[2.2.1]hepta-2,5-diene-2,3-dicarboxylate (55) [7]



$C_{10}H_{10}O_5$: 210.18 g. mol^{-1}

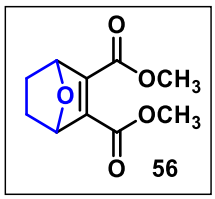
Dimethoxy acetylenedicaroxylate (DMAD) **54** (10g, 70.4 mmol, 1 equiv.) and furan **53** (4.78g, 70.4 mmol, 1 equiv.), was heated in a sealed tube at 100 ^oC for 16 h. Then the crude reaction mixture was directly subjected to the flash column chromatography and (silica gel, hexane: EtOAc 20:80) afforded the titled compound **55** (7.32g, 45 %) as a waxy liquid.

TLC (EtOAc: Hex; 30:70) R_f = 0.4

^{1}H NMR (400 MHz, $CDCl_3$) δ [ppm]: 3.80 (s, 6H), 5.66 (t, ^4J = 1.0 Hz, 2H), 7.20 (t, ^3J = 1.0 Hz, 2H).

^{13}C NMR (100 MHz, $CDCl_3$) δ [ppm]: 52.4, 85.2, 143.3, 153.1, 163.3.

Dimethyl 7- oxabicyclo[2.2.1]hept-2-ene-2,3-dicarboxylate (56) [7]



$C_{10}H_{12}O_5$: 212.20 g. mol^{-1}

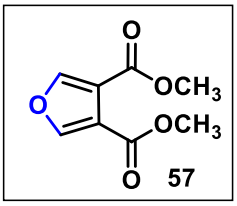
The compound dimethyl 7- oxabicyclo [2.2.1] hepta-2,5-diene-2,3-dicarboxylate **55** (3.5 g, 15.20 mmol) in EtOAc (35 mL) was degassed under reduced pressure for 20 minute using argon medium. After that 10% Pd/C was added to the reaction mixture and again degassed under reduced pressure for 10 minutes afterwards reaction mixture was flushed with H_2 gas. Then the reaction was stirred for 5-6 h under H_2 gas monitored by TLC till starting material consumption. The reaction mixture was then filtered through celite and washed with EtOAc. Then the filtrate was concentrated on a rotatory evaporator and dried under a high vacuum. Flash column chromatography (silica gel, hexane: EtOAc 80:20) afforded the titled compound **56** (2.9 g, 85%) as a colourless liquid.

TLC (EtOAc: Hex; 20:80) R_f = 0.8

^{1}H NMR (400 MHz, $CDCl_3$) δ [ppm]: 1.45 (dd, ^{3}J = 11.7, 3.8 Hz, 2H), 1.98–1.93 (m, 2H), 3.80 (s, 6H,), 5.24 (dd, ^{3}J = 3.0, 1.5 Hz, 2H).

^{13}C NMR (100 MHz, $CDCl_3$) δ [ppm]**:** 24.4, 52.3, 80.7, 143.3, 163.1.

Dimethyl furan-3,4-dicarboxylate (57) [7]



$C_8H_8O_5$: 184.14 g. mol^{-1}

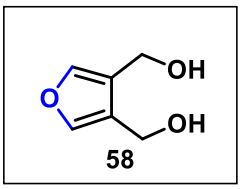
Diester **56** (6.3 g, 27.10 mmol) was heated to 180 °C for 1 h until the evolution of ethylene gas was ceased. The minimum temperature for ethylene evolution was 175 °C. The reaction is monitored by TLC. Flash column chromatography (silica gel, hexane: EtOAc 35:65) furnish the titled compound **57** (6.1 g, 96%) as a colourless liquid.

TLC (EtOAc: Hex: 30:70) R_f = 0.6

^{1}H NMR (400 MHz, $CDCl_3$) δ [ppm]: 3.81 (s, 6H), 7. 90 (s, 2H).

^{13}C NMR (100 MHz, $CDCl_3$) δ [ppm]: 52.0, 118.2, 148.8, 162.1.

Furan-3,4-diyldimethanol (58) [8]



$C_6H_8O_3$: 128.12 g. mol^{-1}

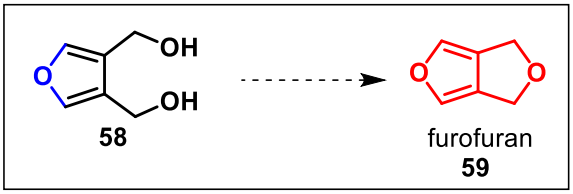
To a cold, (0 °C) suspension of LAH (1.027 g, 28.26 mmol, 2.6 equiv.) in anhydrous diethyl ether (80 mL) was added with stirring dimethyl 3, 4-furandicarboxylate **57** (2.0 g, 10.86 mmol, 1 equiv.). The reaction was stirred at (0 °C) and warmed to room temperature while stirring and then quenched sequentially by slow addition of NH_4Cl solution, water, EtOAc. The reaction mixture was stirred and filtered through celite washed with EtOAc. From the filtrate, the organic layer was separated, and the aqueous layer was extracted with EtOAc. The combined organic layer was dried over (Na_2SO_4) was concentrated on the rotatory evaporator

and dried under a high vacuum. The crude product was purified by column chromatography over silica gel (EtOAc: Hex 35:70) to accomplish the titled compound **58** (1.1 g, 80%) as a colourless oil.

TLC (EtOAc: hexane 50:50) R_f = 0.8

^{1}H NMR (400 MHz, acetone-d_6) δ [ppm]: 4.19 (s, 2H), 4.52 (s, 4H), 7.43 (s, 2H).

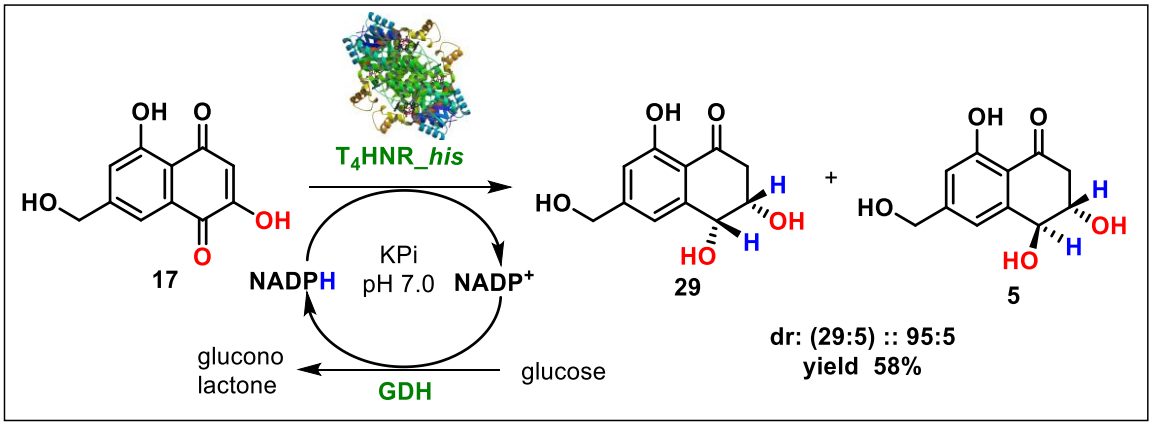
^{13}C NMR (100 MHz, acetone-d_6) δ [ppm]: 55.4, 126.5, 141.3.



Entry	Reagents	Observation
1	MnO_2, Et_3SiH, TFA, DCM, (-5^{o}C to rt)	No desired product
2	DMC, NaOMe, CH_3CN	No desired product
3	BuLi, TsCl, BuLi, heat	No desired product
4	Ag_2O, TsCl, KI, rt	No desired product
5	Et_3N, MsCl, DCM, rt	No desired product
6	NaH, $PO(OMe)_3$, rt, 24 h	No desired product

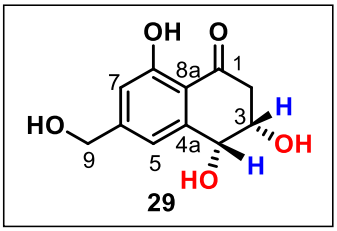
VI. Enzyme catalyzed reduction of synthesized substrates

a) Reduction of 2,5-dihydroxy-7-(hydroxymethyl)naphthalene-1,4-dione (17) using T4HNR_*his* to *cis*-nodulone D (29)



Scheme S5. Reduction of compound **17** using wild-type T_4HNR

(3*S*,4*R*)-3,4,8-trihydroxy-6-(hydroxymethyl)-3,4-dihydronaphthalenone (*cis*-nodulone D, 29)



$C_{11}H_{12}O_5$: 224.06 g.mol^{-1}

Procedure: To a buffer solution (20 mL, KPi = 50 mM, EDTA =1.0 mM, DTT = 1.0 mM), current of argon was bubbled for half an hour under reduced pressure for 30 min. Then, glucose (81.8 mg, 0.45 mmol, 5 equiv.), $NADP^+$ (7.15 mg, 0.009 mmol, 0.1 equiv., 10 %) and glucose dehydrogenase (500 µL, 175U, 350 U/mL) were added and the mixture was stirred slowly under argon at rt. After 15 min, the substrate, hydroxynaphthoquinone **17** (20 mg, 0.090 mmol, 1 equiv.) in 2-propanol (1 mL, 5 % v/v) was added slowly, while stirring. At last, 1,3,6,8-tetrahydroxynaphthalene reductase (1.5 mL, 8 U/mL, 12 U) was added and the mixture was stirred at rt. After 24 h, the solution was acidified with 10 % of H_2SO_4 (0.5 mL) and EtOAc (10 mL) was added and stirred vigorously, precipitating the enzyme. The solution was filtered with sintered glass with a silica bed and washed with EtOAc (20 mL). The aqueous layer was extracted (2×) with EtOAc and the combined organic layer was washed with brine (30 mL). The organic layer was dried over Na_2SO_4, filtered and the solvent was removed under reduced

pressure to afford a dark brown residue, which was purified by using column chromatography (Silica gel, 100-200 mess size, DCM: MeOH, 9:1) to afford desired product as a distereomeric mixture of **29** and **5** (95:5) in yield 58 % (12 mg) as a yellowish solid.

TLC (chloroform/methanol, 9:1 v/v): R_f = 0.16;

^{1}H NMR (400 MHz, acetone-d_6): δ (ppm) 2.91 (d, ^3J = 4.8 Hz, 2H, 2-CH_2), 4.35 – 4.39 (m, 1H, H-3), 4.65 (s, 2H, 9 -CH_2), 4.89 (d, 1H, ^3J = 2.6 Hz, H-4), 6.86 (s, 1H, CH_{ar}), 7.14 (s, 1H, CH_{ar}), 12.39 (s, 1H, OH-8).

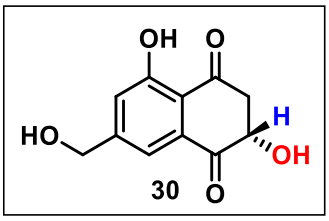
^{13}C NMR (100 MHz, acetone-d_6): δ (ppm) 44.1, 64.2, 70.5, 70.9, 114.1, 115.1, 117.2, 145.8, 153.4, 163.1, 203.9.

CD (*c* = 2.0 mM, *l* = 0.1 mm, methanol): λ [nm] (CD mdeg) 202 (4.64), 207 (18.92), 214 (32.45), 222 (16.21), 227 (–2.94), 232 (–8.39), 241 (–2.74), 252 (–10.97), 265 (–20.85), 277 (–10.46), 287 (–1.04), 317 (2.69), 339 (2.81), 369 (0.04).

$[\alpha]_D^{27}$ = – 2.99° (c = 0.07 g/100 mL in methanol)

HRMS (ESI) m/z: [M + H]$^+$ 225.0757 (calculated); 225.0766 (found).

(*S*)-2,5-dihydroxy-7-(hydroxymethyl)-2,3-dihydronaphthalene-1,4-dione (30)



$C_{11}H_{10}O_5$: 222.20 g.mol^{-1}

Procedure: To a buffer solution (10 mL, KPi = 50 mM, EDTA =1.0 mM, DTT = 1.0 mM), current of argon was bubbled for half an hour under reduced pressure for 30 min. Then, glucose (40.90 mg, 0.227 mmol, 5 equiv.), $NADP^+$ (3.57 mg, 0.0045 mmol, 0.1 equiv., 10 %) and glucose dehydrogenase (250 µL, 45 U, 200 U/mL) were added and the mixture was stirred slowly under argon at rt. After 15 min, the substrate, **17** (10 mg, 0.045 mmol, 1 equiv.) in 2-propanol (500 µL, 5 % v/v) was added slowly, while stirring. At last, 1,3,6,8-tetrahydroxynaphthalene reductase (750 µL, 8 U/mL, 6 U) was added and the mixture was stirred at rt. After 4 h, the solution was acidified with 10 % of H_2SO_4 (0.5 mL) and EtOAc (10 mL) was added and stirred vigorously, precipitating the enzyme. The solution was filtered with

sintered glass with a silica bed and washed with EtOAc (20 mL). The aqueous layer was extracted (2×) with EtOAc and the combined organic layer was washed with brine (30 mL). The organic layer was dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure to afford a dark brown residue, which was purified by using column chromatography (Silica gel, 100-200 mess size, DCM: MeOH, 85:15) to afford compound **30** (3 mg, 30%) as a brownish solid.

TLC (chloroform/methanol, 9:1 v/v): R_f = 0.5;

^{1}H NMR (400 MHz, acetone-*d*$_6$): δ (ppm) 3.17 – 3.34 (m, 2H, H-3), 4.76 (s, 2H, 7-CH_2), 4.90 (dd, *J* = 10.8, 6.4 Hz, 1H, H-2), 7.28 (s, 1H, CH_{ar}), 7.51 (s, 1H, CH_{ar}), 12.13 (s, 1H, OH-8).

^{13}C NMR (100 MHz, acetone-*d*$_6$): δ (ppm) 46.4, 63.7, 72.4, 116.3, 117.9, 120.6, 135.4, 154.2, 162.5, 197.7, 202.2.

HRMS (ESI) m/z: [M + H]$^+$ 223.0601 (calculated); 223.0583 (found).

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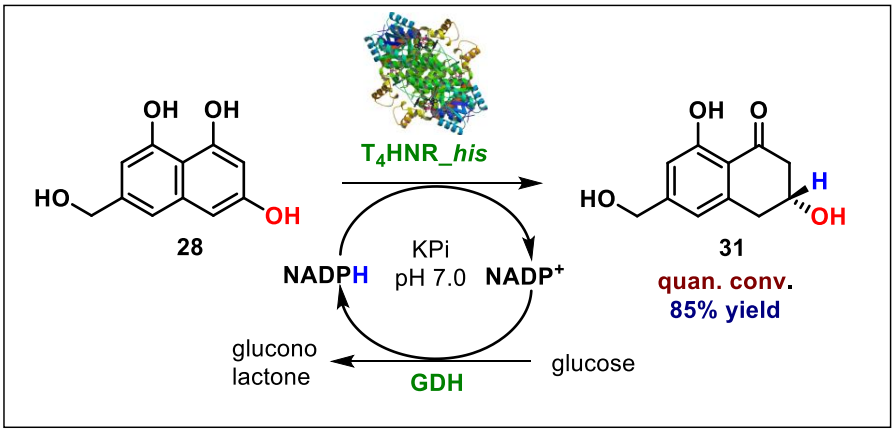
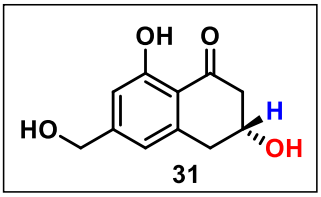
b) Reduction of 6-(hydroxymethyl)naphthalene-1,3,8-triol (28) using T$_4$HNR_*his* to 31.



Scheme S6. Reduction of compound **28** using wild-type T_4HNR

(*R*)-3,8-dihydroxy-6-(hydroxymethyl)-3,4-dihydronaphthalenone (31)



$C_{11}H_{12}O_4$: 208.07 g.mol^{-1}

Procedure: To a buffer solution (25 mL, KPi = 50 mM, EDTA = 1.0 mM, DTT = 1.0 mM), current of argon was bubbled for half an hour under reduced pressure for 30 min. Then, glucose (109.2 mg, 0.606 mmol, 5 equiv.), $NADP^+$ (9.55 mg, 0.012 mmol, 0.1 equiv., 10 %) and glucose dehydrogenase (500 µL, 125U, 250 U/mL) were added and the mixture was stirred slowly under argon at rt. After 15 min, the substrate, **28** (25 mg, 0.121 mmol, 1 equiv.) in 2-propanol (1.25 mL, 5 % v/v) was added slowly, while stirring. At last, 1,3,6,8-tetrahydroxynaphthalene reductase (1.25 mL, 8 U/mL, 10 U) was added and the mixture was stirred at rt. After 24 h, the solution was acidified with 10 % of H_2SO_4 (0.5 mL) and EtOAc (10 mL) was added and stirred vigorously, precipitating the enzyme. The solution was filtered with sintered glass with a silica bed and washed with EtOAc (20 mL). The aqueous layer was extracted (2×) with EtOAc and the combined organic layer was washed with brine (30 mL). The organic layer was dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure to afford a dark brown residue, which was purified by using column chromatography (Silica gel, 100-200 mess size, DCM: MeOH, 85:15) to afford compound **31** (21 mg, 85%) as a white solid.

TLC (chloroform/methanol, 85:15 v/v): R_f = 0.46;

^{1}H NMR (400 MHz, acetone-d_6): δ (ppm) 2.71 (dd, ^2J = 15.7 Hz, ^3J = 2.3 Hz, 1H, H-2), 2.95–2.99 (m, 2H, H-2, H-3), 3.19 (dd, ^2J = 16.8 Hz, ^3J = 7.0 Hz, 1H, H-3), 4.39 (br s, 1H, OH-9), 4.41 – 4.45 (m, 1H, H-3), 4.62 (d, J = 5.0 Hz, 2H, 9 -CH_2), 6.77 (s, 2H, CH_{ar}), 12.40 (s, 1H, OH-8).

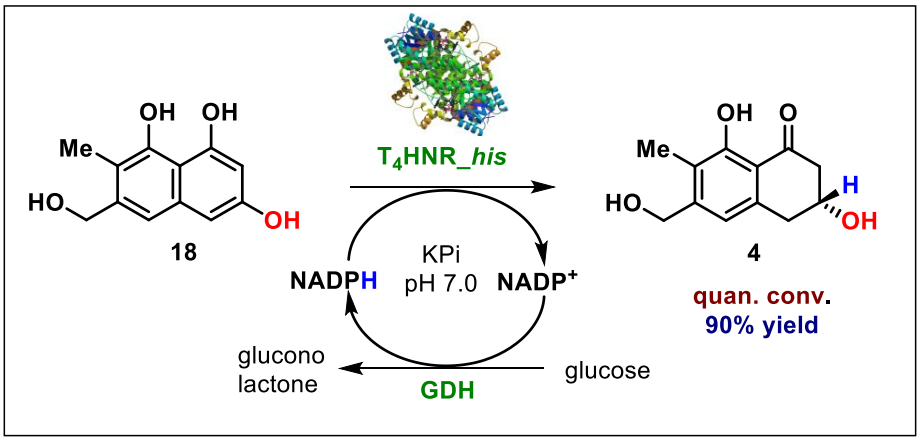
^{13}C NMR (100 MHz, acetone-d_6): δ (ppm) 39.0, 47.8, 64.0, 66.5, 113.0, 116.4, 118.2, 143.6, 153.1, 163.5, 204.6.

CD (c = 2.0 mM, l = 0.1 mm, methanol): λ [nm] (CD mdeg) 212 (1.60), 221 (–1.38), 225 (–5.04), 230 (–7.43), 237 (-4.91), 245 (–2.16), 255 (–3.69), 272 (–4.49), 290 (–0.67), 330 (3.34), 356 (0.48).

$[\alpha]_D^{27}$ = – 14 ^{o} (c = 0.1 g/100 mL in methanol)

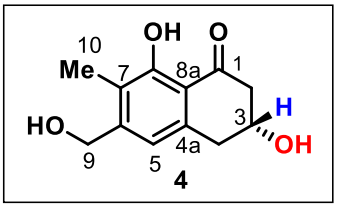
HRMS (ESI) m/z: [M + H]$^+$ 209.0808 (calculated); 209.0806 (found).

c) Reduction of 6-(hydroxymethyl)-7-methylnaphthalene-1,3,8-triol (18) using T$_4$HNR_*his* to nodulone C (4)



Scheme S7. Reduction of compound **18** using wild-type T$_4$HNR

(*R*)-3,8-dihydroxy-6-(hydroxymethyl)-7-methyl-3,4-dihydronaphthalenone (nodulone C, 4)



C$_{12}$H$_{14}$O$_4$: 222.24 g.mol^{-1}

Procedure: To a buffer solution (10 mL, KPi = 50 mM, EDTA = 1.0 mM, DTT = 1.0 mM), current of argon was bubbled for half an hour under reduced pressure for 30 min. Then, glucose (40.9 mg, 0.22 mmol, 5 equiv.), NADP$^+$ (3.57 mg, 0.0045 mmol, 0.1 equiv., 10 %) and glucose dehydrogenase (150 µL, 45U, 285 U/mL) were added and the mixture was stirred slowly under argon at rt. After 15 min, the substrate, **18** (10 mg, 0.045 mmol, 1 equiv.) in 2-propanol (500 µL, 5 % v/v) was added slowly, while stirring. At last, 1,3,6,8-tetrahydroxynaphthalene reductase (500 µL, 8 U/mL, 4 U) was added and the mixture was stirred at rt. After 24 h, the solution was acidified with 10 % of H_2SO_4 (0.5 mL) and EtOAc (10 mL) was added and stirred vigorously, precipitating the enzyme. The solution was filtered with sintered glass with a silica bed and washed with EtOAc (20 mL). The aqueous layer was extracted (2×) with EtOAc and the combined organic layer was washed with brine (10 mL). The organic layer was dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure to afford a dark brown

residue, which was purified by using column chromatography (Silica gel, 100-200 mess size, DCM: MeOH, 9:1) to afford compound **4** (9.0 mg, 90 %) as a white solid.

TLC (chloroform/methanol, 9:1 v/v): R_f = 0.46;

^{1}H NMR (400 MHz, methanol-d_4): δ (ppm) 2.11 (s, 3H, CH_3), 2.69 (dd, ^2J = 17.0 Hz, ^3J = 7.7 Hz, 1H, H-2), 2.90–2.96 (m, 2H, H-2, H-4), 3.18 (dd, ^2J = 16.0 Hz, ^3J = 3.4 Hz, 1H, H-4), 4.28 – 4.33 (m, 1H, H-3), 4.61 (s, 2H, 9 -CH_2), 6.90 (s, 1H, CH_{ar}).

^{13}C NMR (100 MHz, methanol-d_4): δ (ppm) 9.7, 38.9, 47.9, 62.9, 67.2, 116.3, 118.9, 122.1, 140.4, 149.8, 161.3, 204.9.

CD (*c* = 2.0 mM, *l* = 0.1 mm, methanol): λ [nm] (CD mdeg) 203 (–0.64), 210 (0.48), 219 (–2.58), 227 (–6.15), 236 (–3.34), 245 (–0.92), 261 (–3.41), 289 (–1.14), 318 (0.94), 341 (1.99), 371 (0.26).

$[\alpha]_D^{27}$ = – 4.49^o (c = 0.07 g/100 mL in methanol)

HRMS (ESI) m/z: $[M + H]^+$ 223.0965 (calculated); 223.0955 (found).

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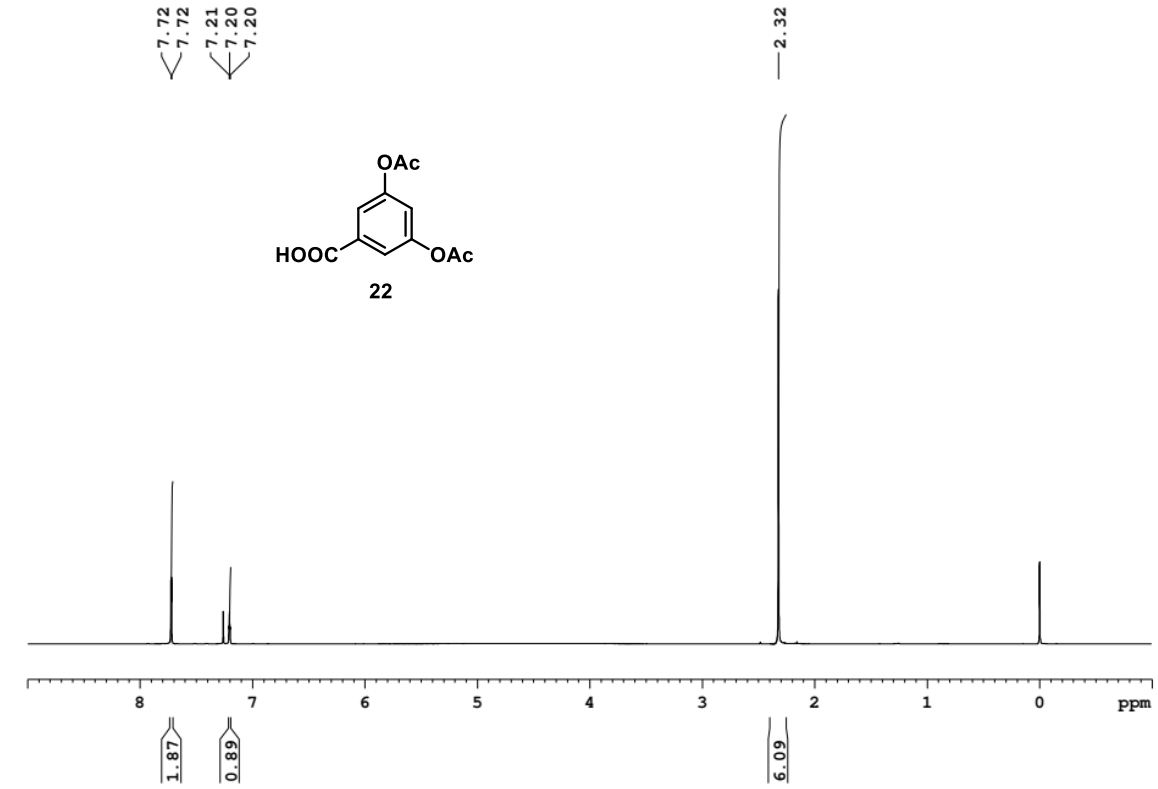
^{1}H NMR and ^{13}C NMR spectroscopic data from reference [9] compared to those of synthetic 3,8-dihydroxy-6-(hydroxymethyl)-7-methyl-3,4-dihydronaphthalenone.

Proton ^{1}H	**Natural (*R*)-3,8-dihydroxy-6-(hydroxymethyl)-7-methyl-3,4-dihydronaphthalenone (400 MHz, methanol-d_4) δ (ppm)**	**Synthetic (*R*)-3,8-dihydroxy-6-(hydroxymethyl)-7-methyl-3,4-dihydronaphthalenone (400 MHz, methanol-d_4) δ (ppm)**
H-2	2.70 (ddd, *J* = 16.8, 7.8, 1.2 Hz, 1H)	2.69 (d, *J* = 17.0, 7.6 Hz, 1H)
	2.93 (ddd, *J* = 16.8, 3.6, 1.2 Hz, 1H)	2.96 – 2.90 (m, 2H, H-2, H-4)
H-3	4.31 (dddd, *J* = 7.8, 7.8, 3.6, 3.6 Hz, 1H)	4.28 – 4.33 (m, 1H)
H-4	2.94 (dd, *J* = 15.6, 7.8 Hz, 1H)	3.18 (dd, *J* = 16.0, 3.4 Hz, 1H)
	3.19 (dd, *J* = 15.6, 3.6 Hz, 1H)	
H-5	6.91 (s, 1H)	6.90 (s, 1H)
H-9	4.62 (s)	4.61 (s)
H-10	2.12 (s)	2.11 (s)

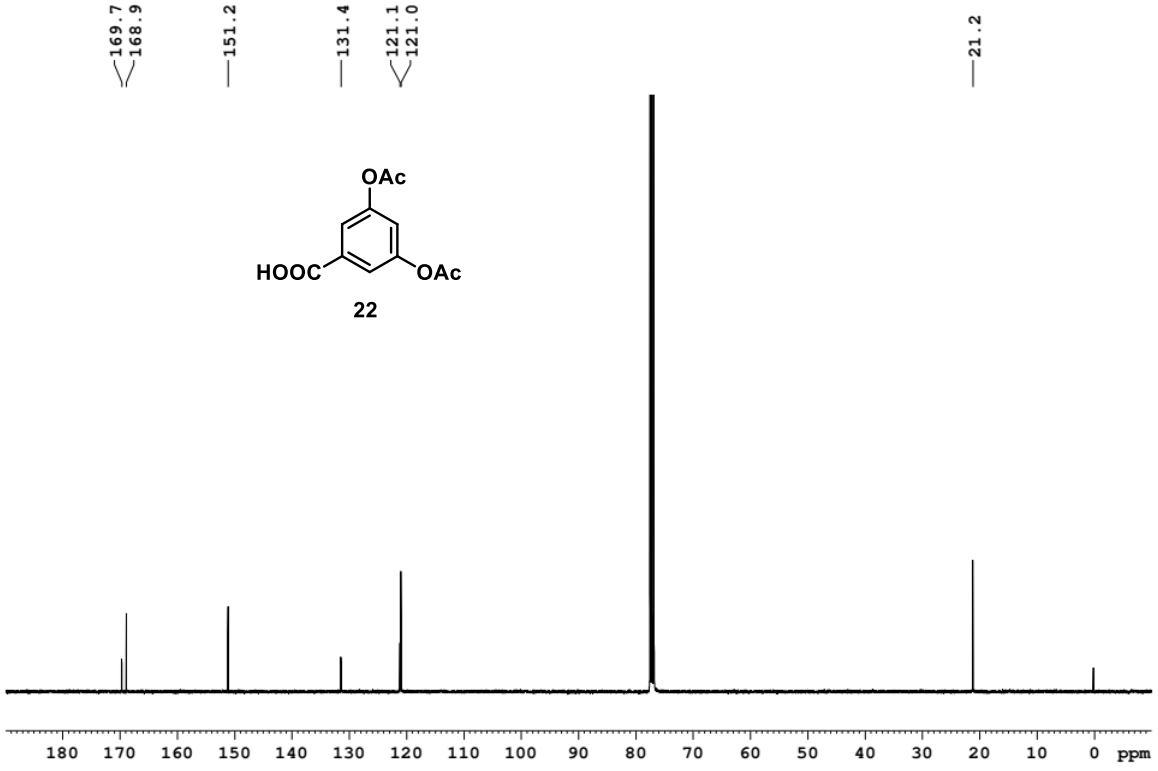
^{13}C	**Natural (*R*)-3,8-dihydroxy-6-(hydroxymethyl)-7-methyl-3,4-dihydronaphthalenone (100 MHz, methanol-d_4) δ (ppm)**	**Synthetic (*R*)-3,8-dihydroxy-6-(hydroxymethyl)-7-methyl-3,4-dihydronaphthalenone (100 MHz, methanol-d_4) δ (ppm)**
C-1	205.1	204.9
C-2	48.0	47.9
C-3	67.3	67.2
C-4	39.1	38.9
C-4a	140.6	140.4
C-5	119.1	118.9
C-6	149.5	149.8
C-7	122.2	122.1
C-8	161.5	161.3
C-8a	116.4	116.3
C-9	63.0	62.9
C-10	9.8	9.7

VII. NMR Spectra

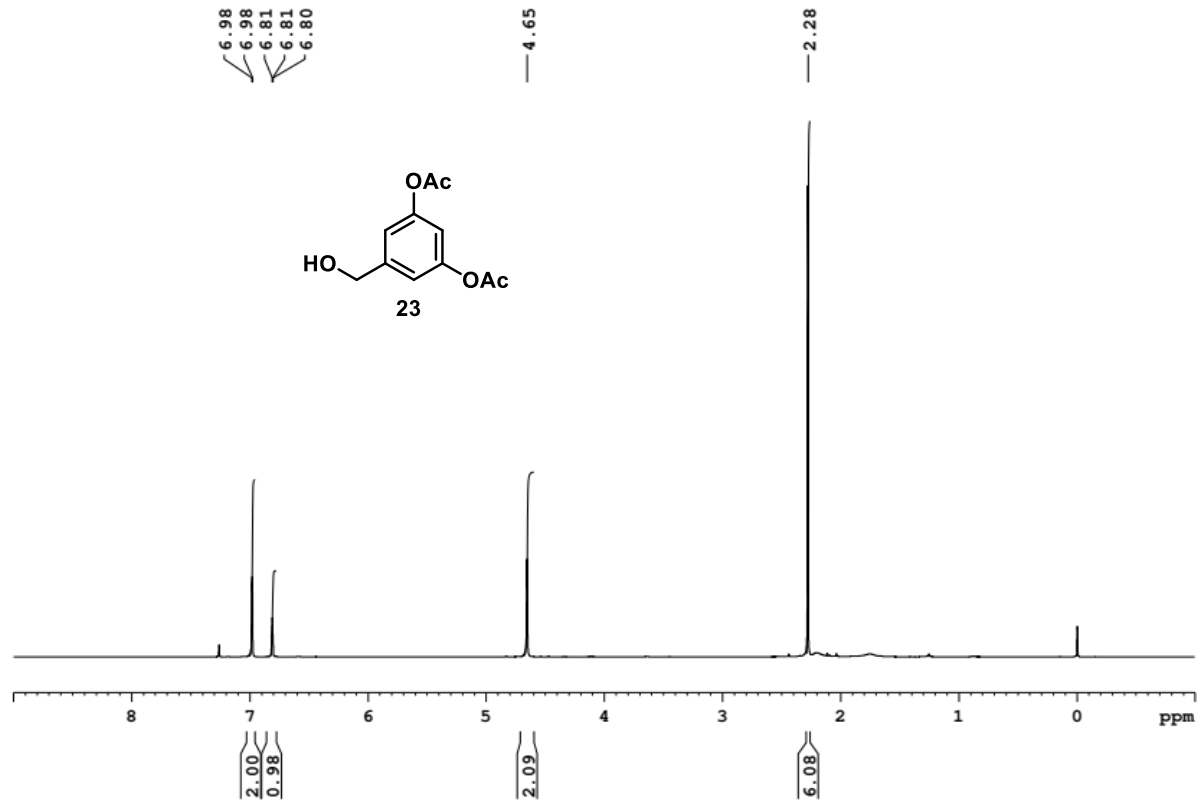
^{1}H NMR (400 MHz, $CDCl_3$)



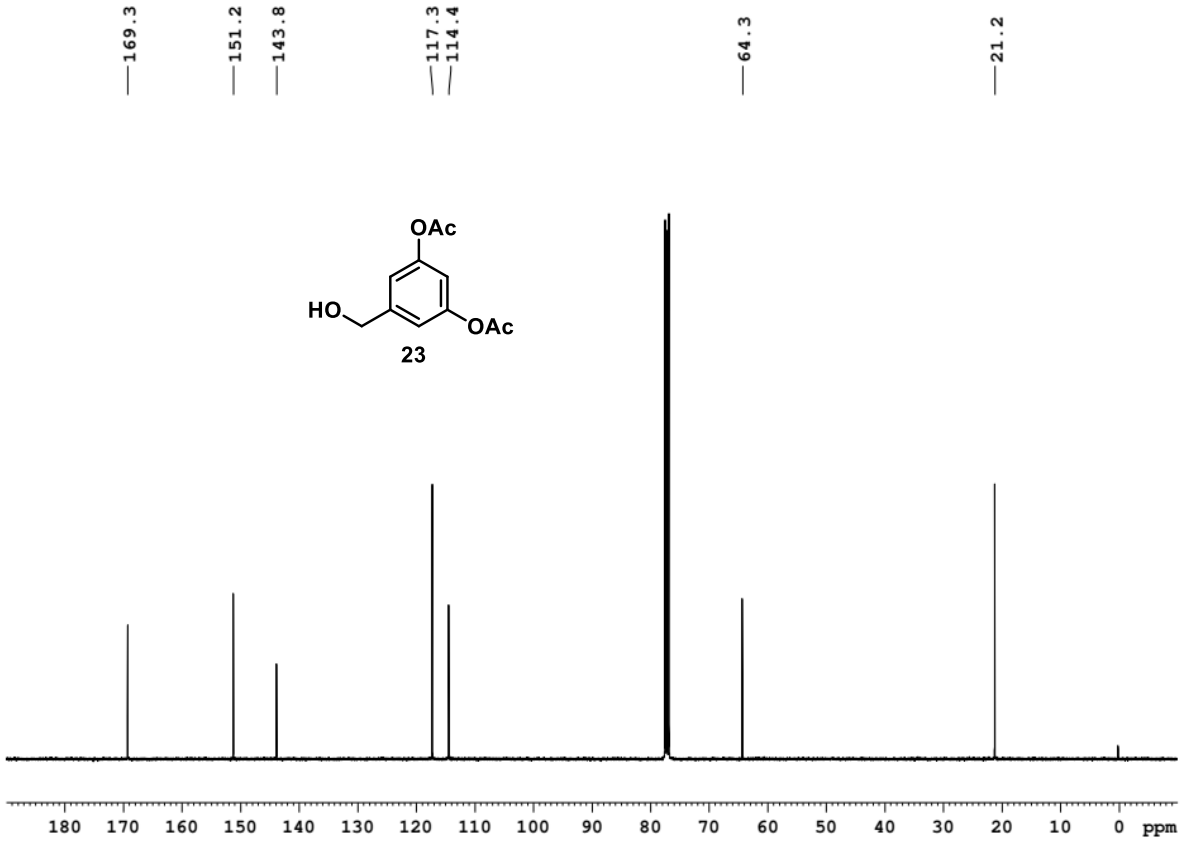
^{13}C NMR (100 MHz, $CDCl_3$)



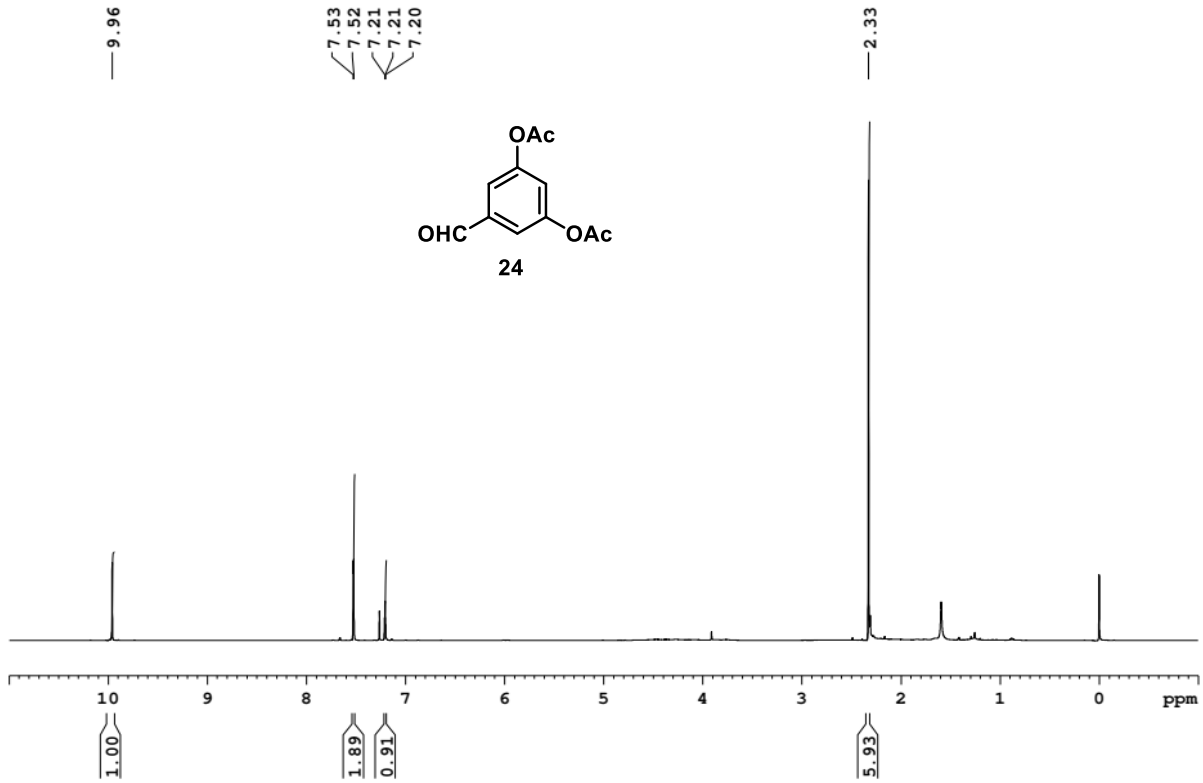
^{1}H NMR (400 MHz, $CDCl_3$)



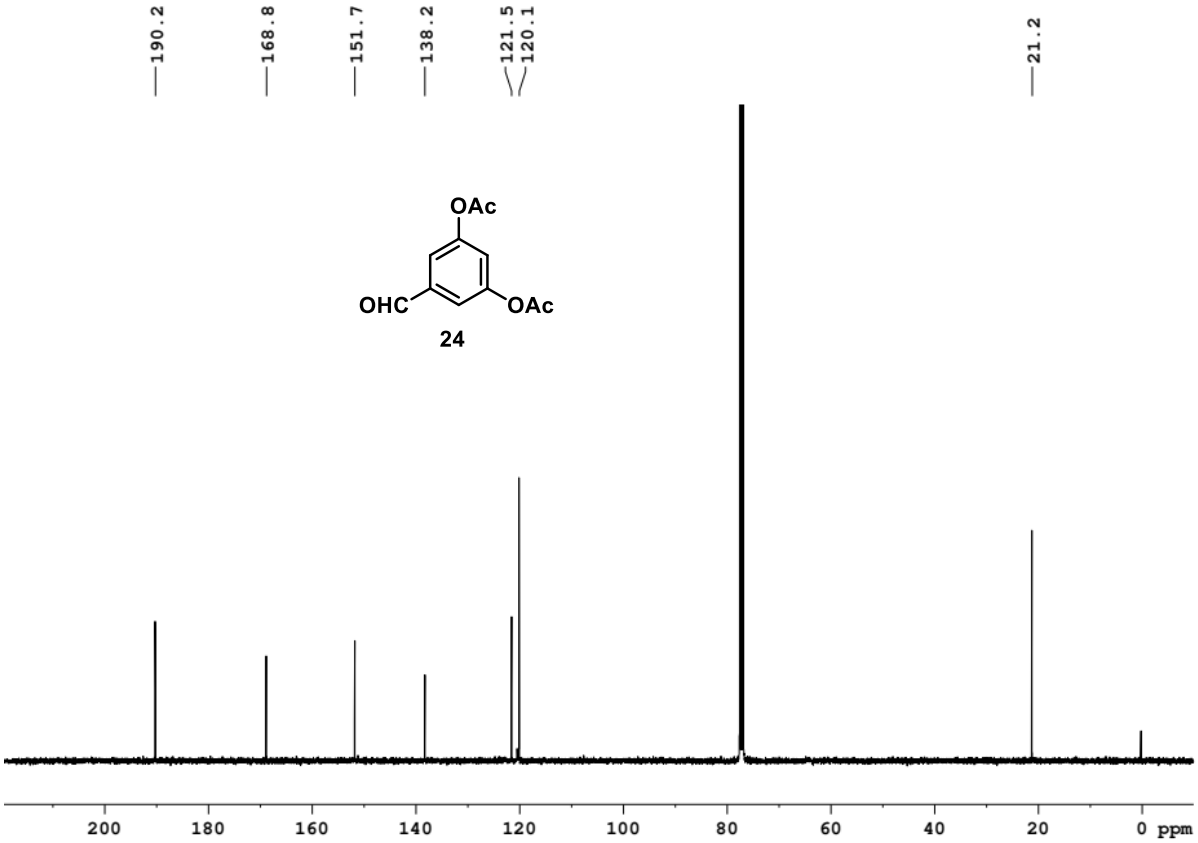
^{13}C NMR (100 MHz, $CDCl_3$)



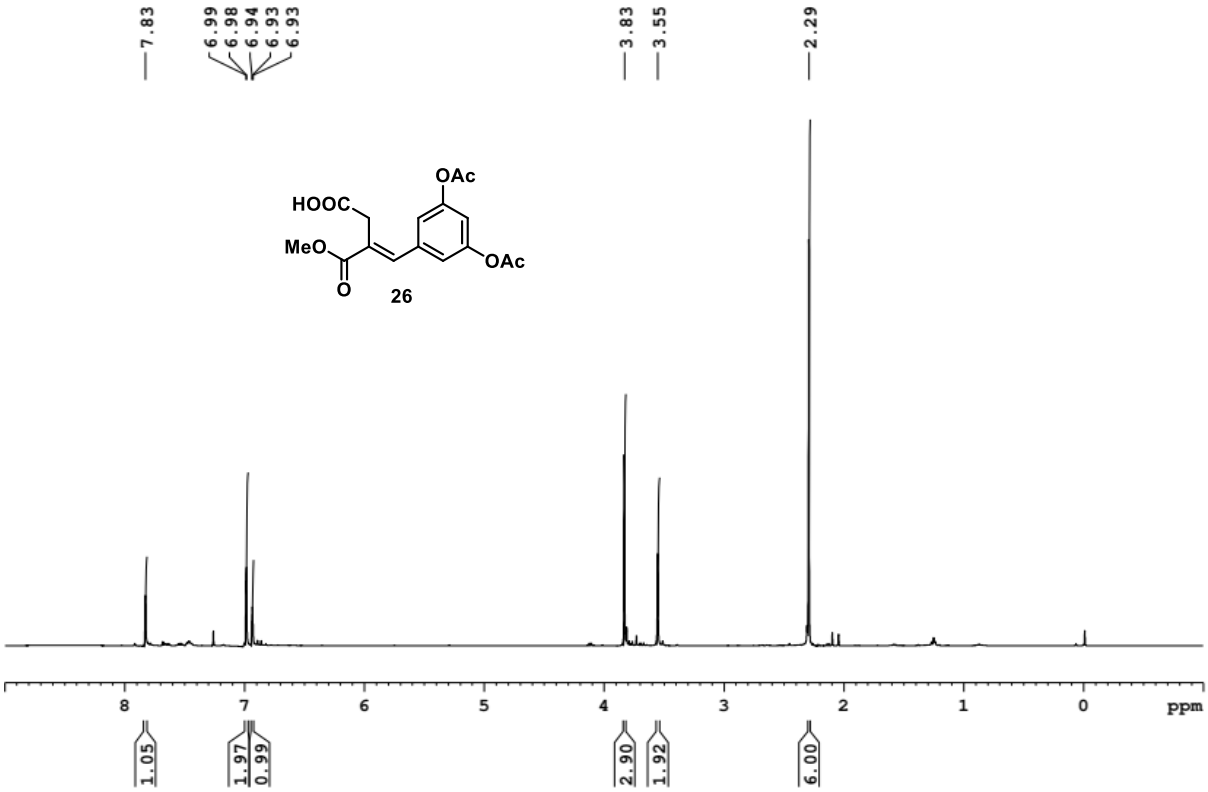
^{1}H NMR (400 MHz, $CDCl_3$)



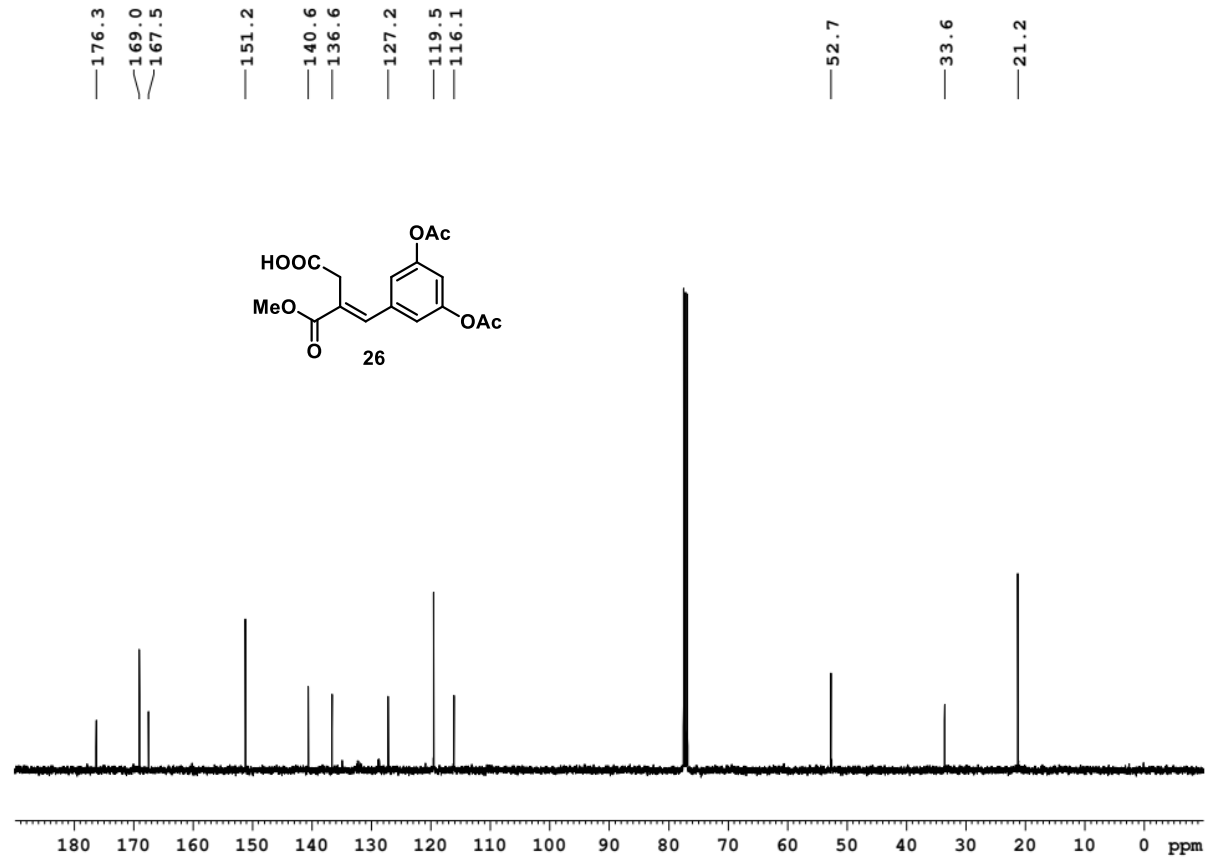
^{13}C NMR (100 MHz, $CDCl_3$)



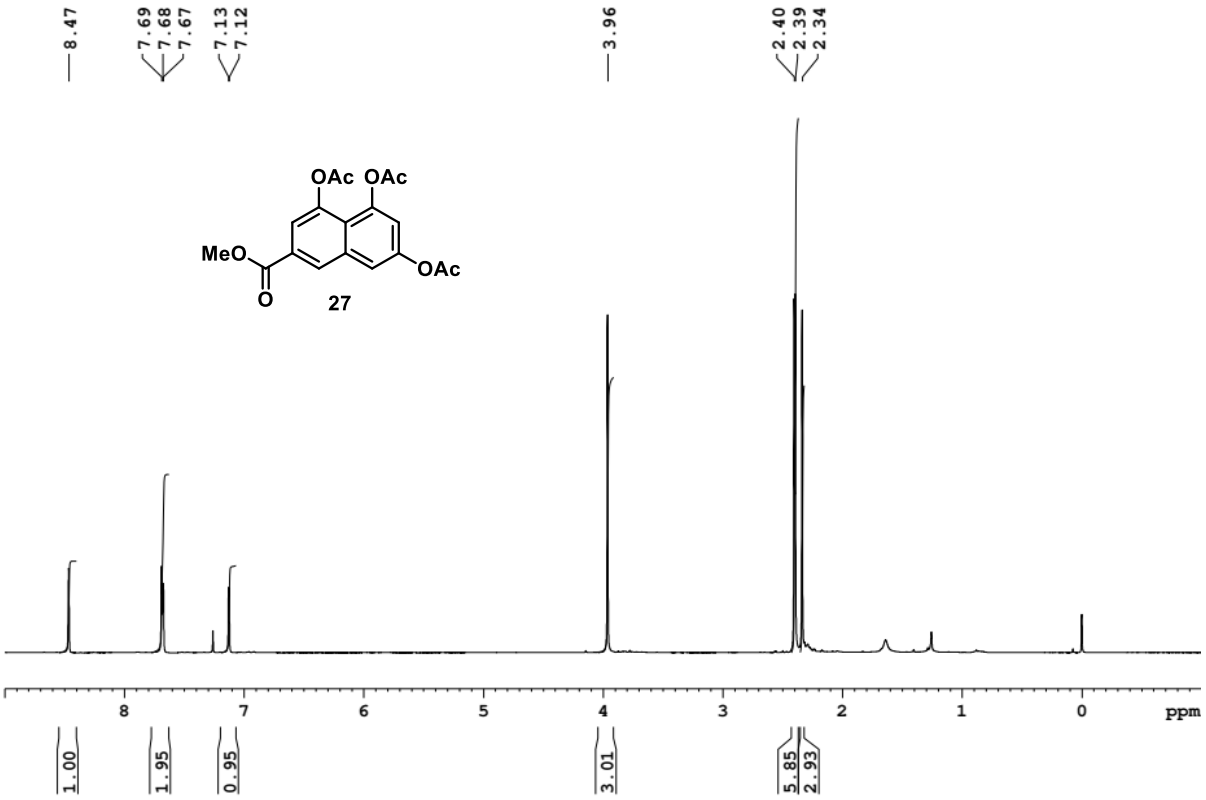
^{1}H NMR (400 MHz, $CDCl_3$)



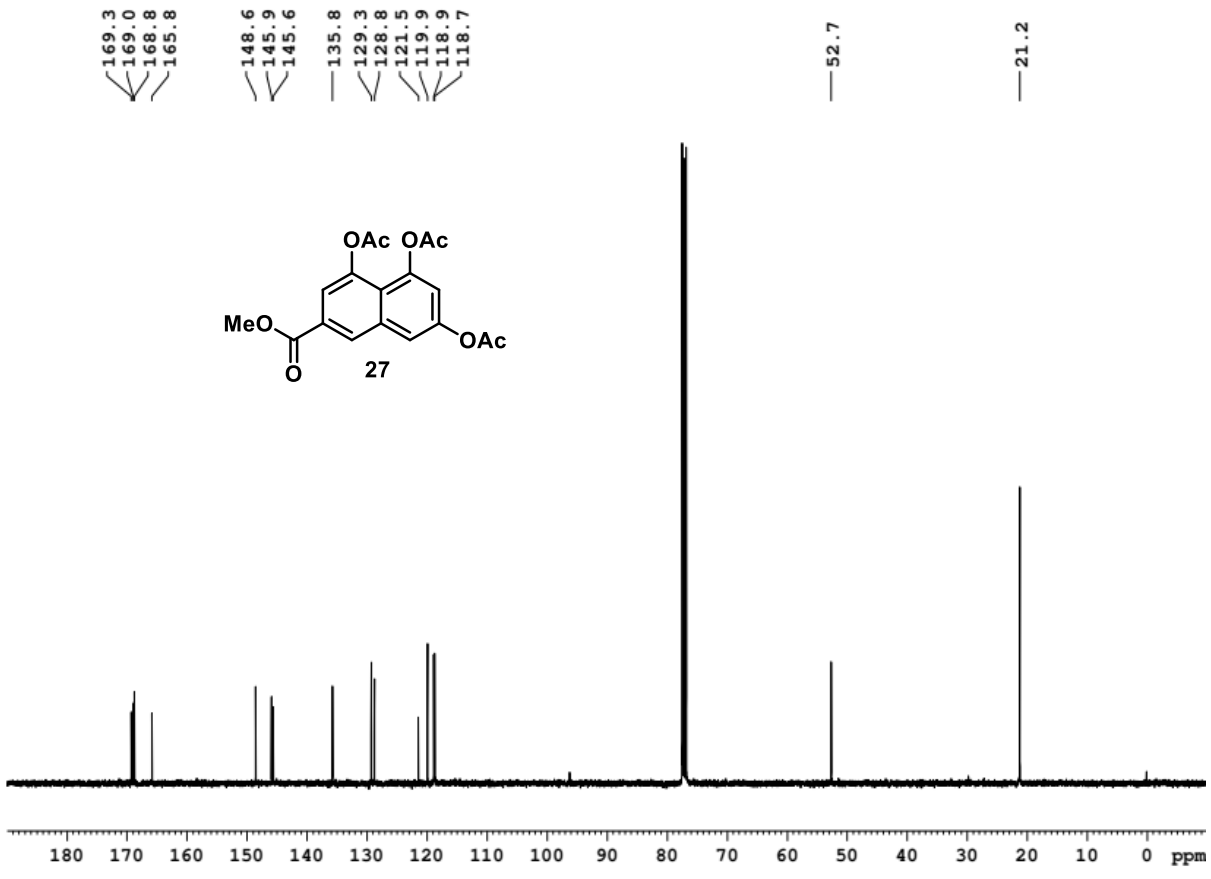
^{13}C NMR (100 MHz, $CDCl_3$)



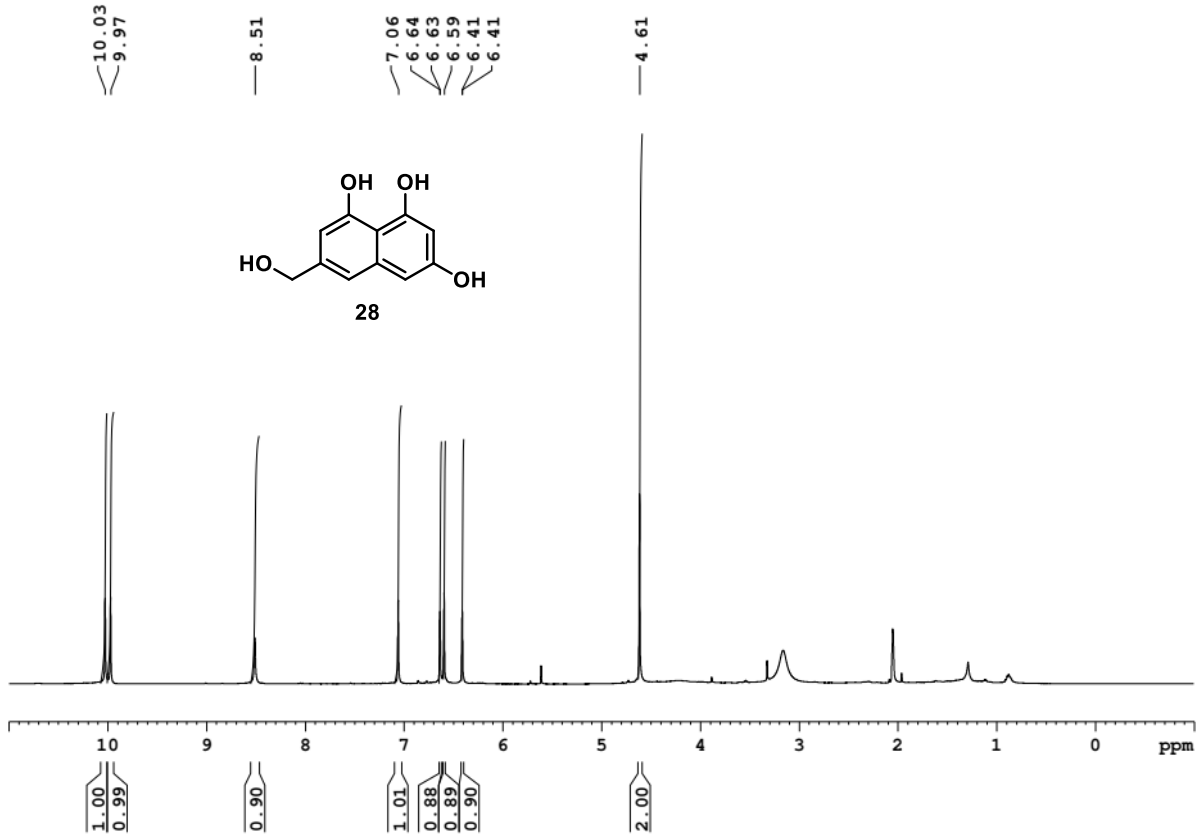
^{1}H NMR (400 MHz, $CDCl_3$)



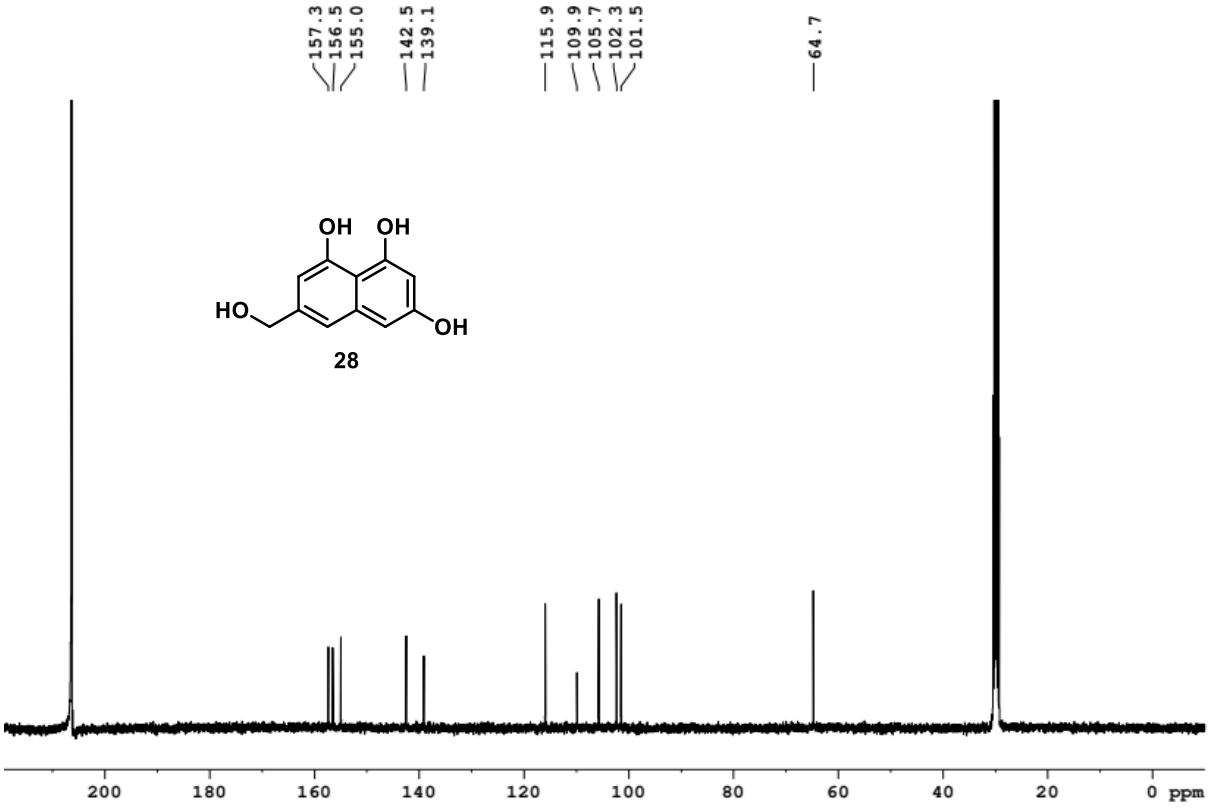
^{13}C NMR (100 MHz, $CDCl_3$)



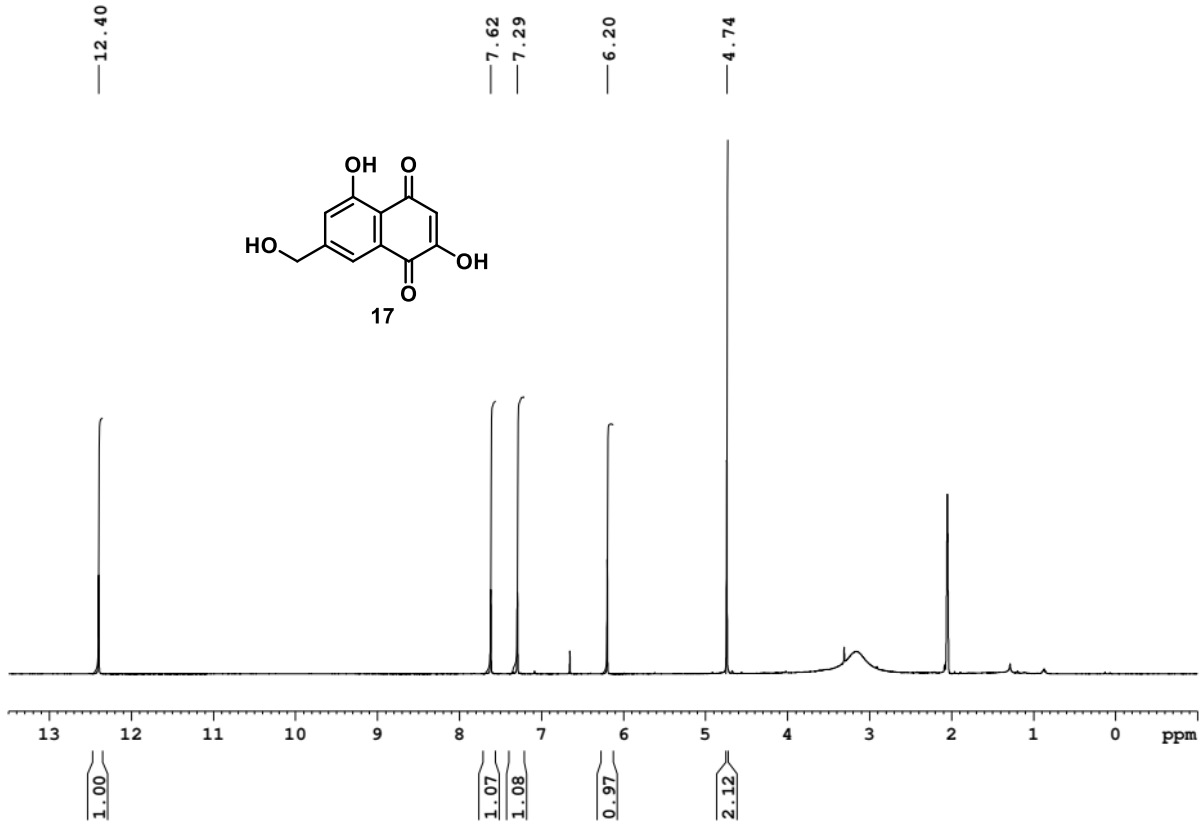
^{1}H NMR (400 MHz, acetone-d_6)



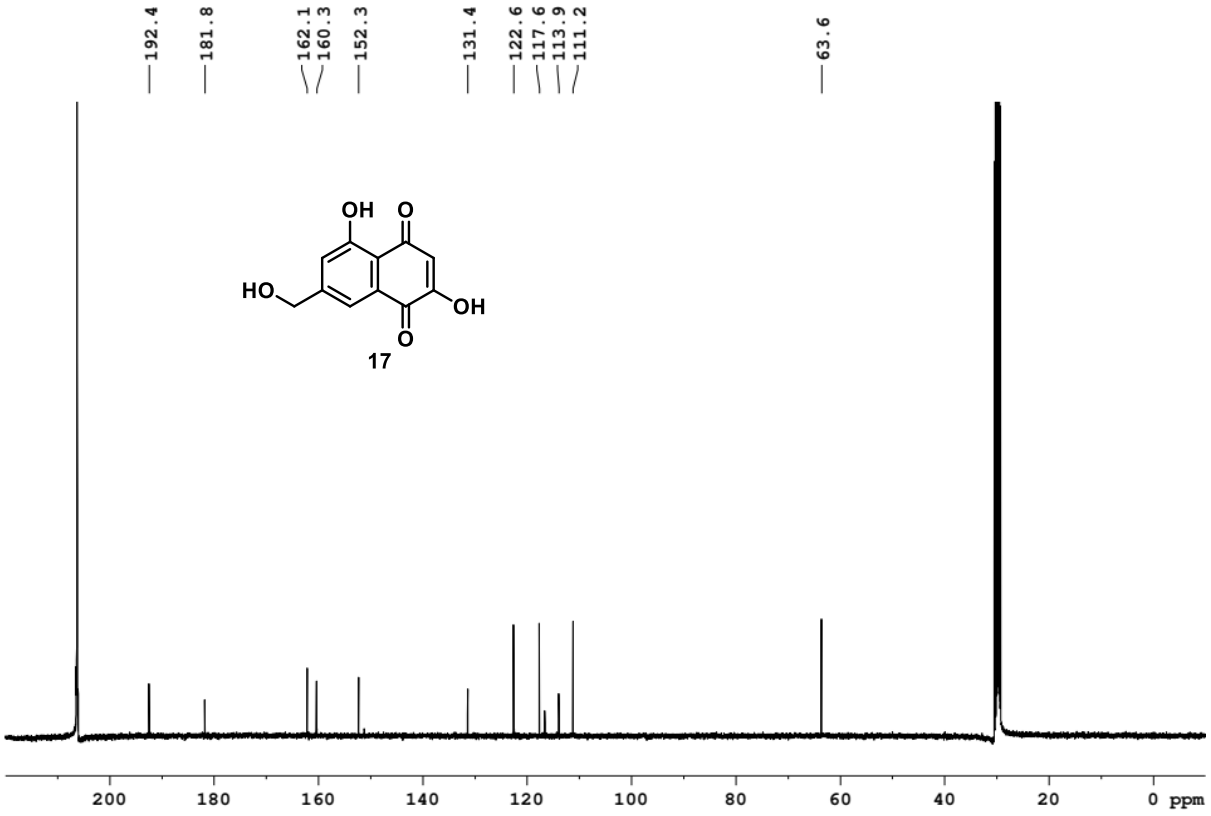
^{13}C NMR (100 MHz, acetone-d_6)



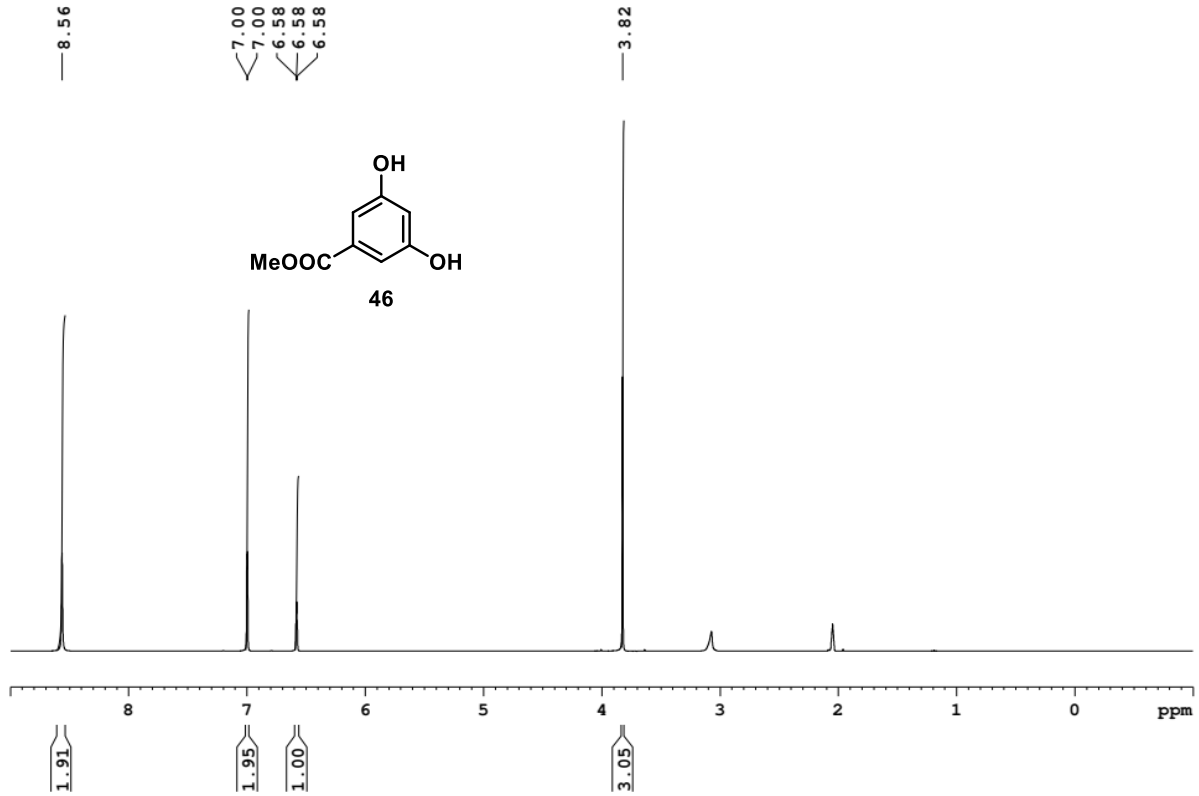
^{1}H NMR (400 MHz, acetone-d_6)



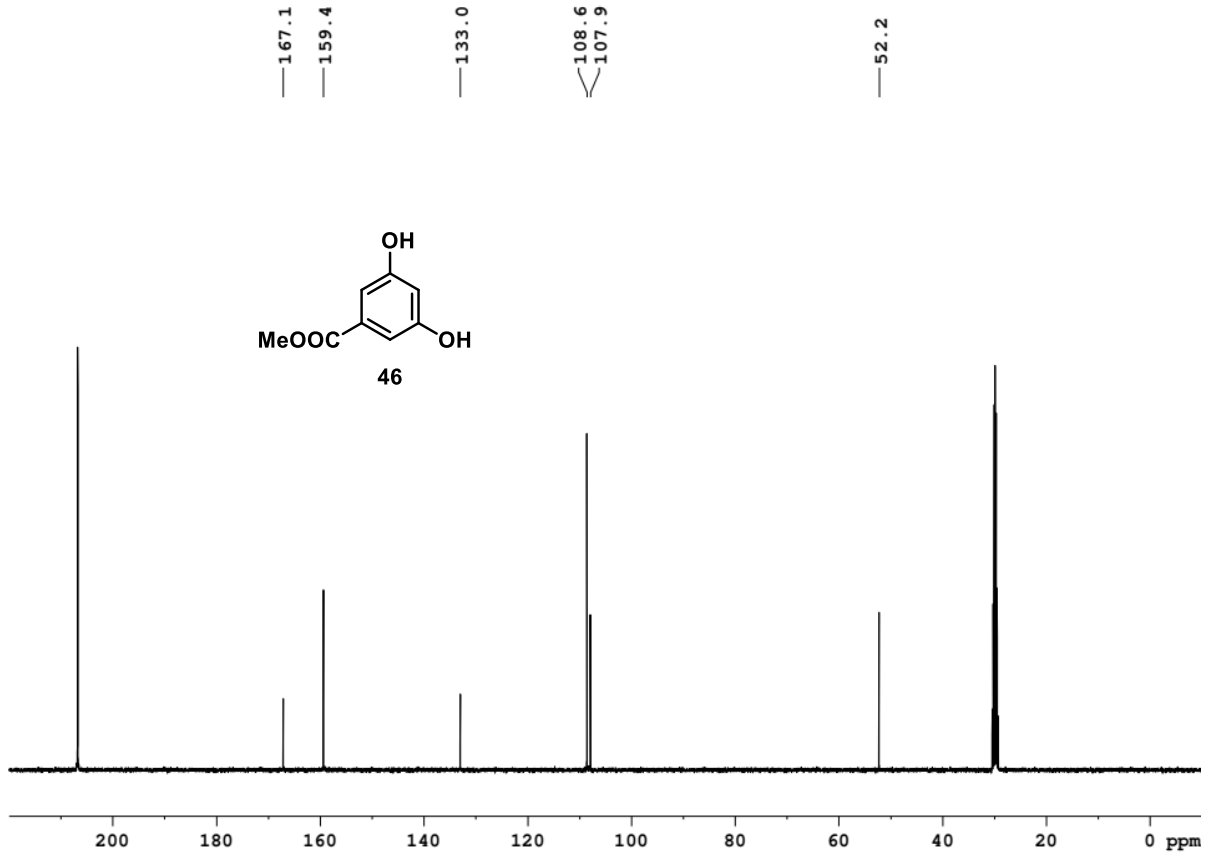
^{13}C NMR (100 MHz, acetone-d_6)



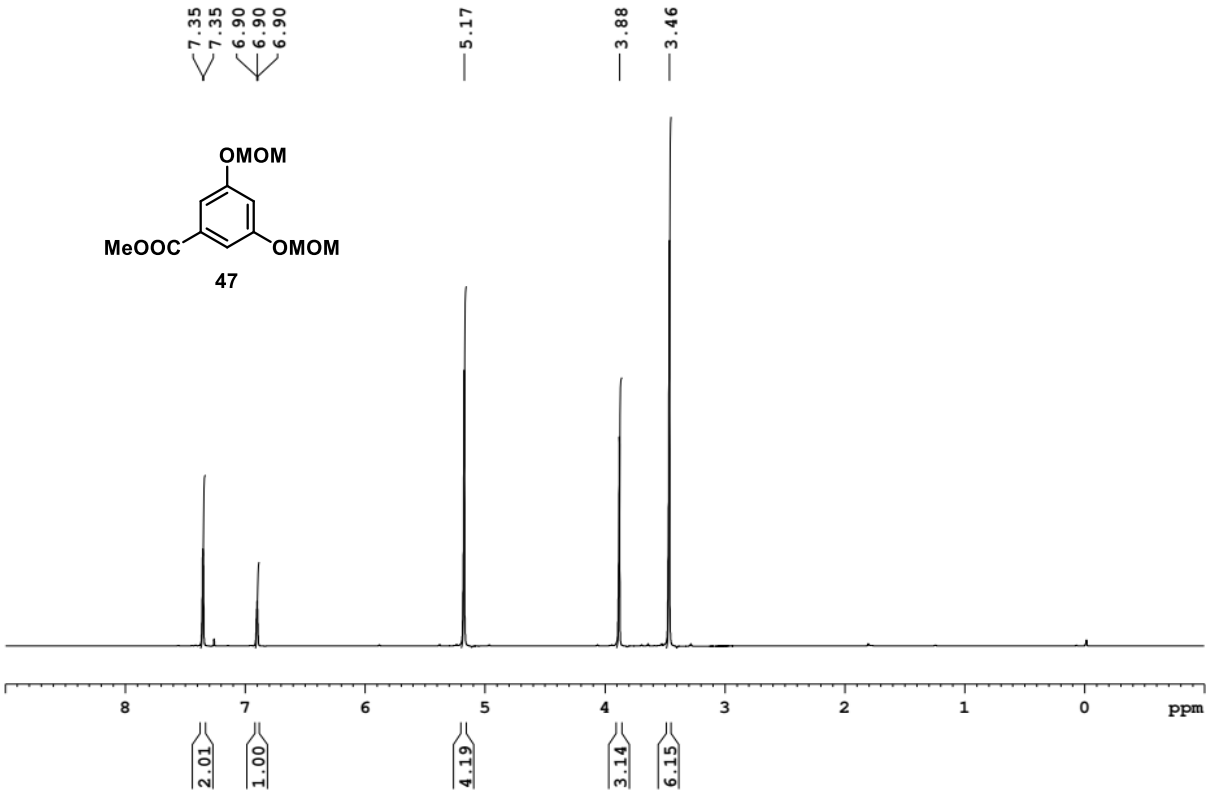
^{1}H NMR (400 MHz, acetone-d_6)



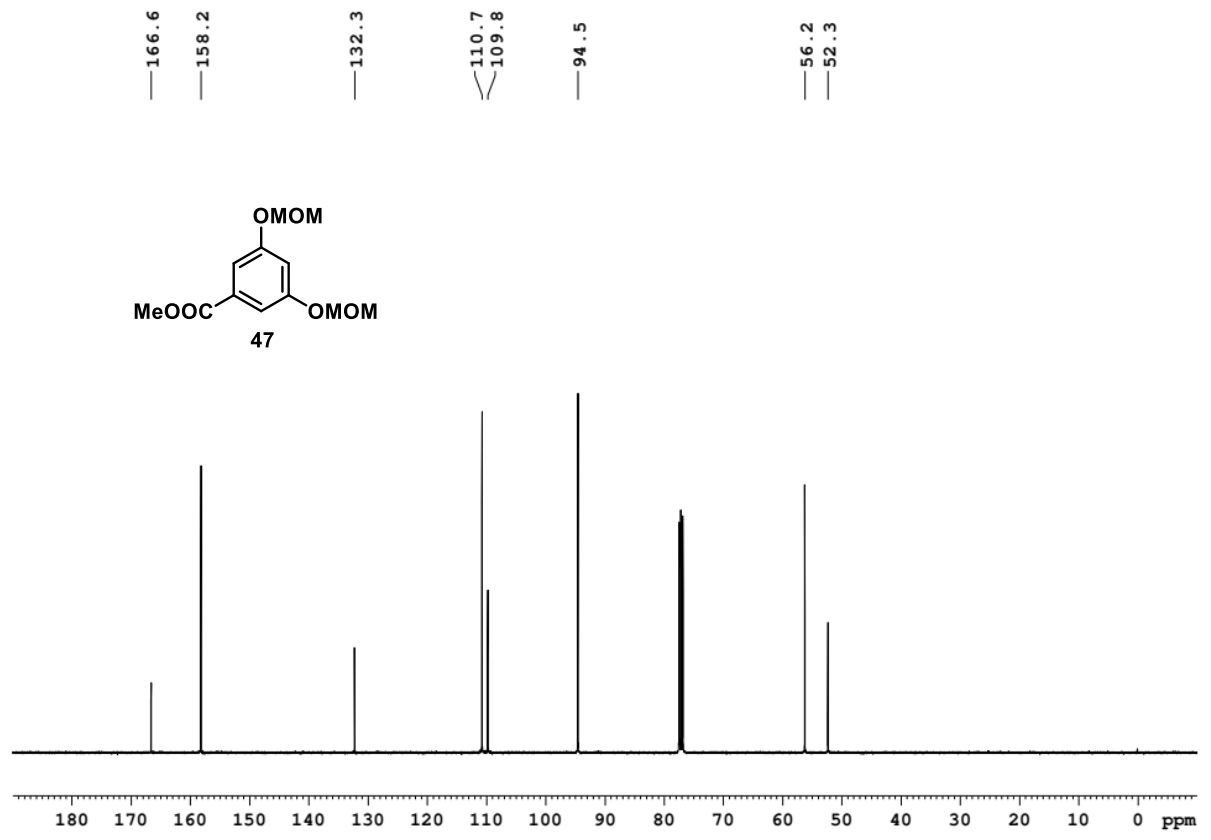
^{13}C NMR (100 MHz, acetone-d_6)



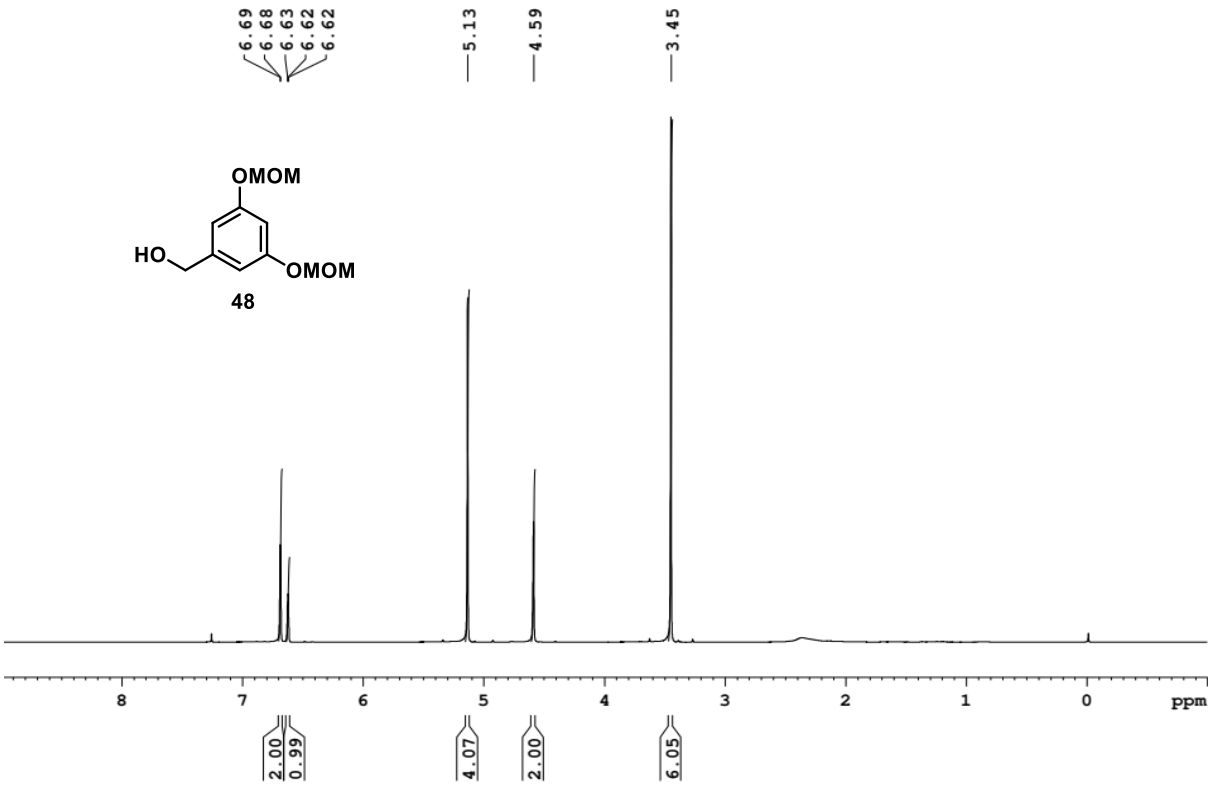
^{1}H NMR (400 MHz, $CDCl_3$)



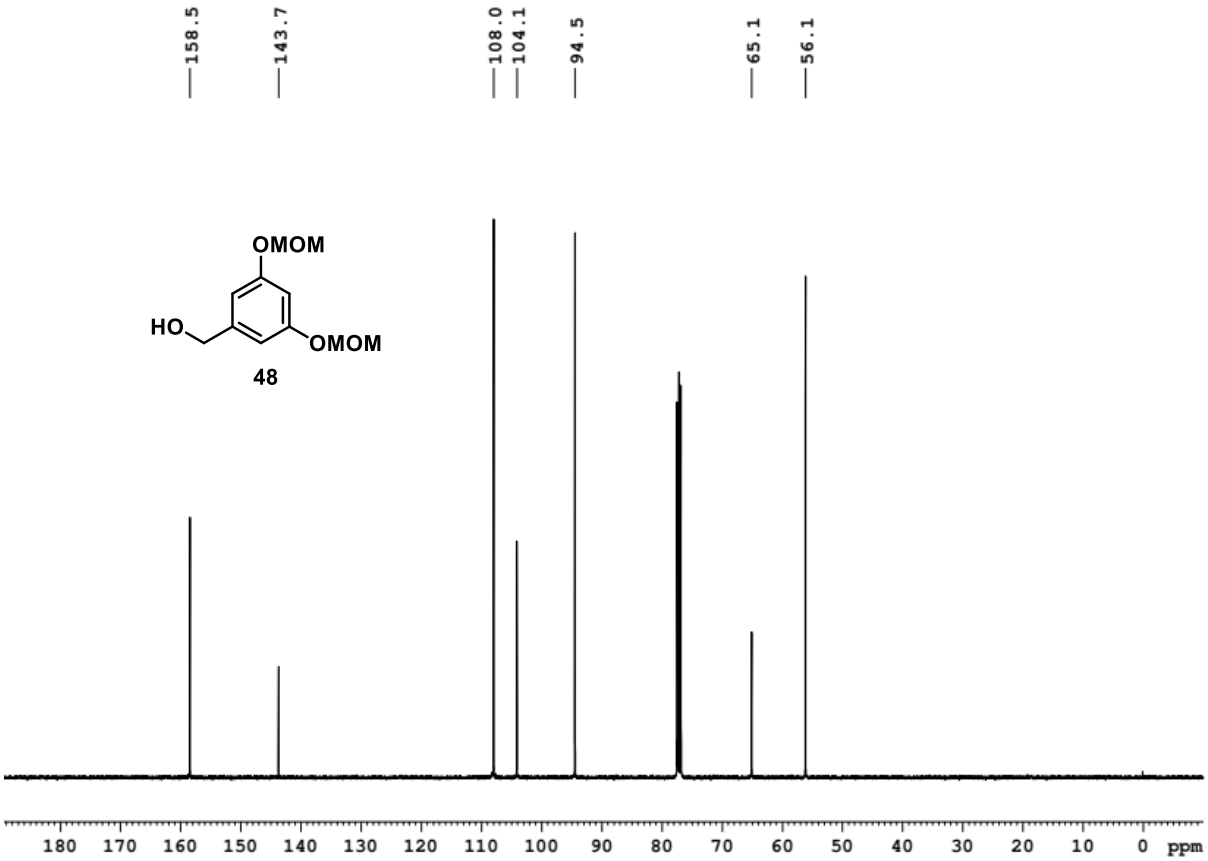
^{13}C NMR (100 MHz, $CDCl_3$)



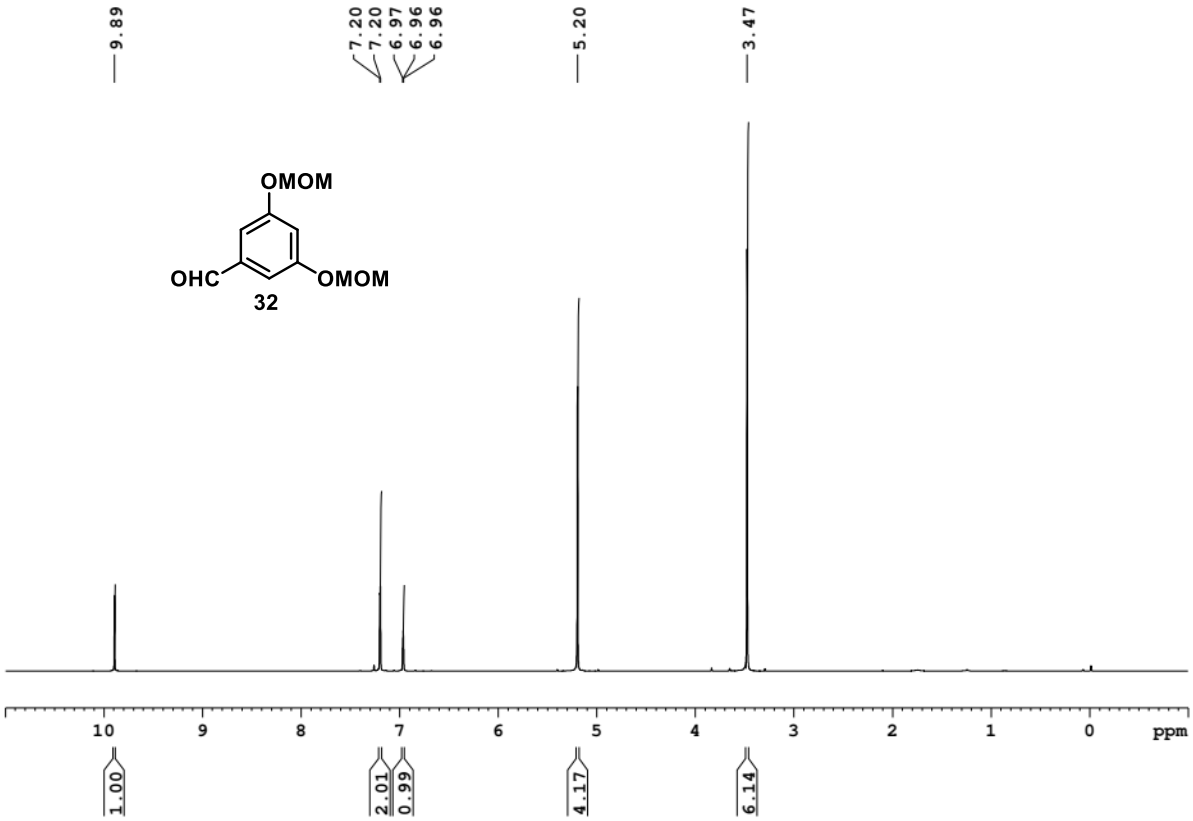
^{1}H NMR (400 MHz, $CDCl_3$)



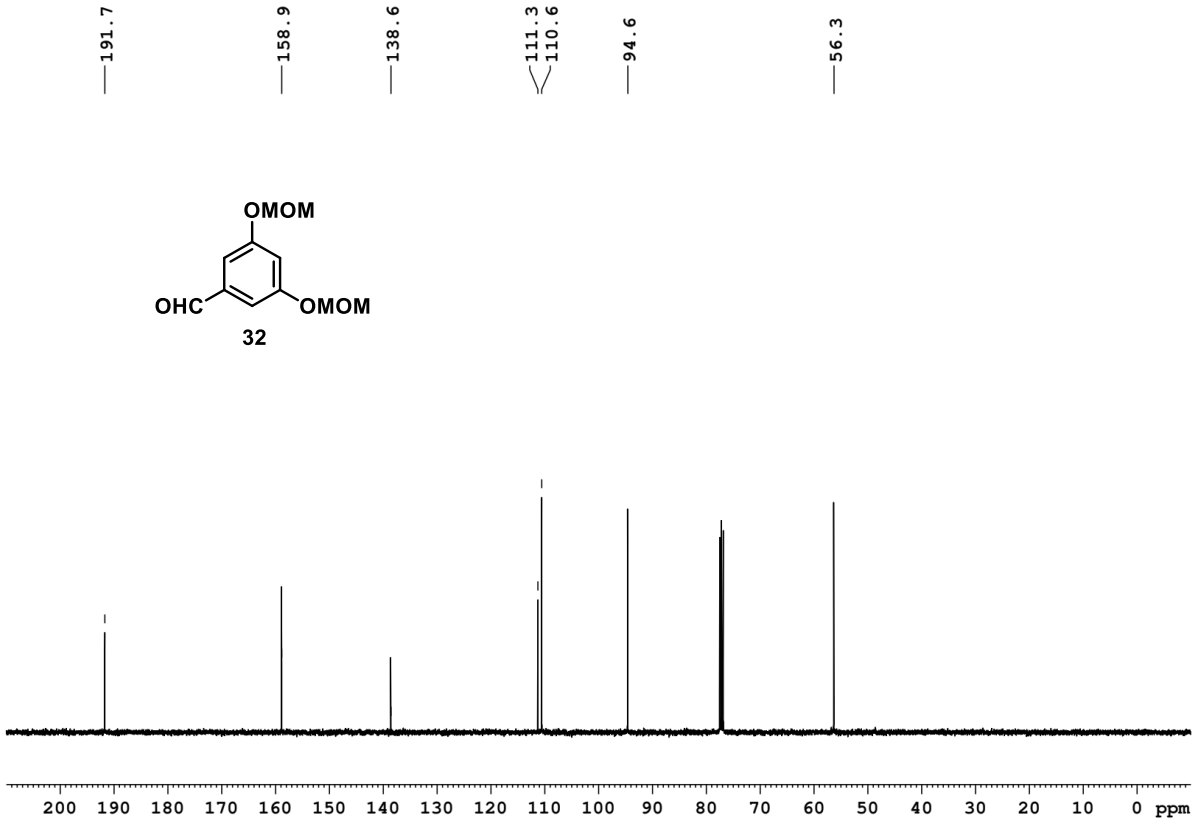
^{13}C NMR (100 MHz, $CDCl_3$)



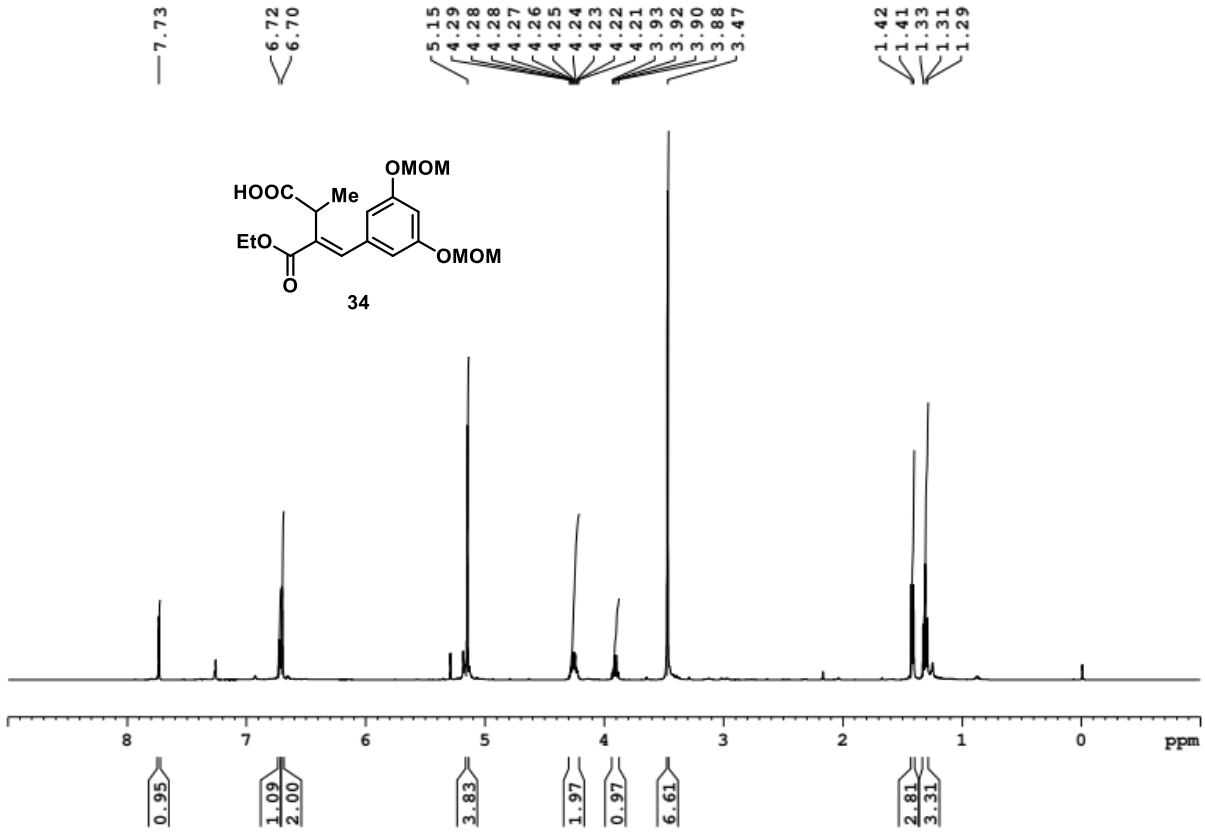
^{1}H NMR (400 MHz, $CDCl_3$)



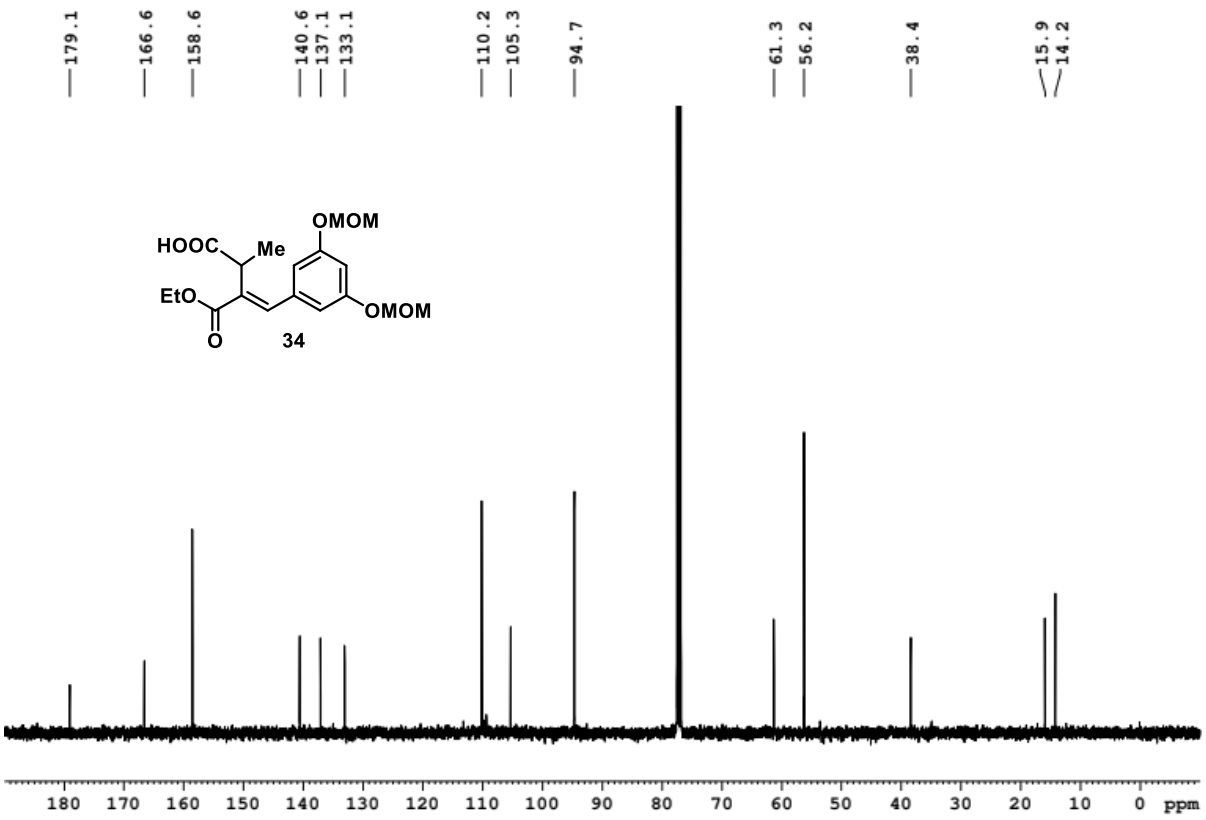
^{13}C NMR (100 MHz, $CDCl_3$)



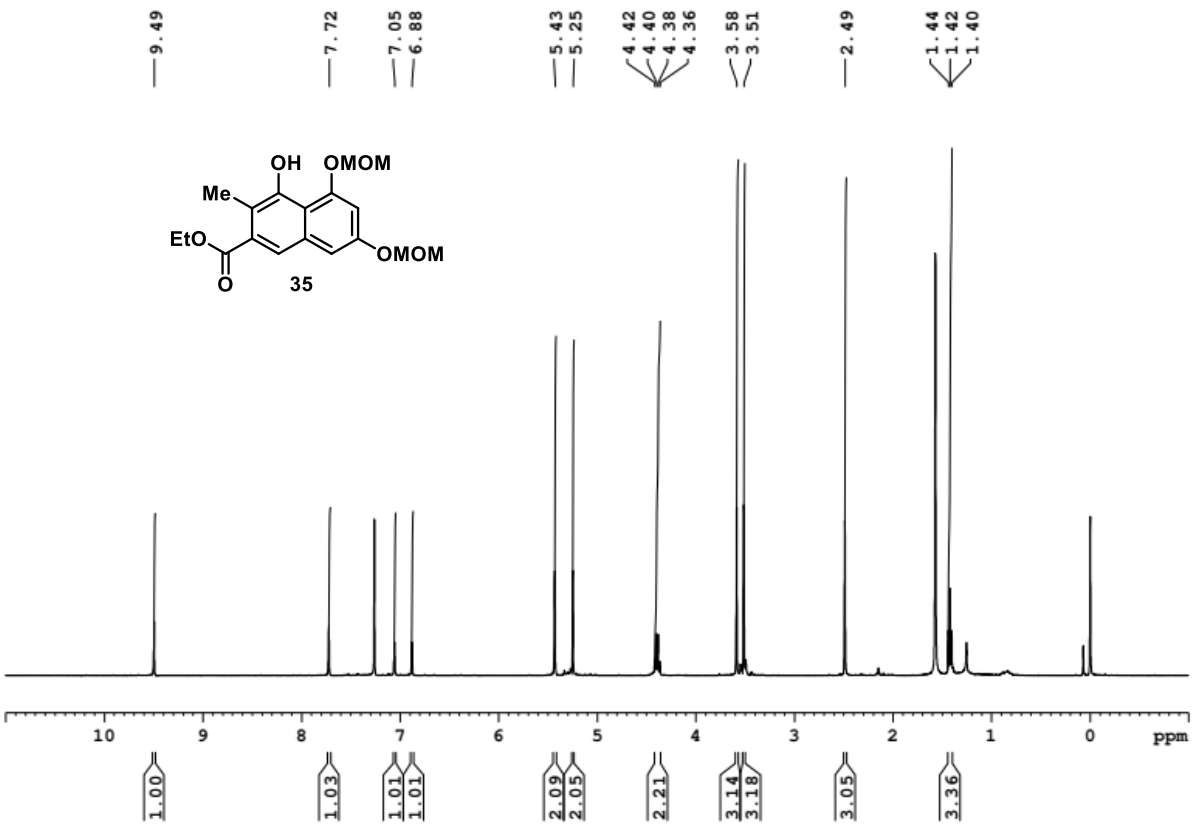
^{1}H NMR (400 MHz, $CDCl_3$)



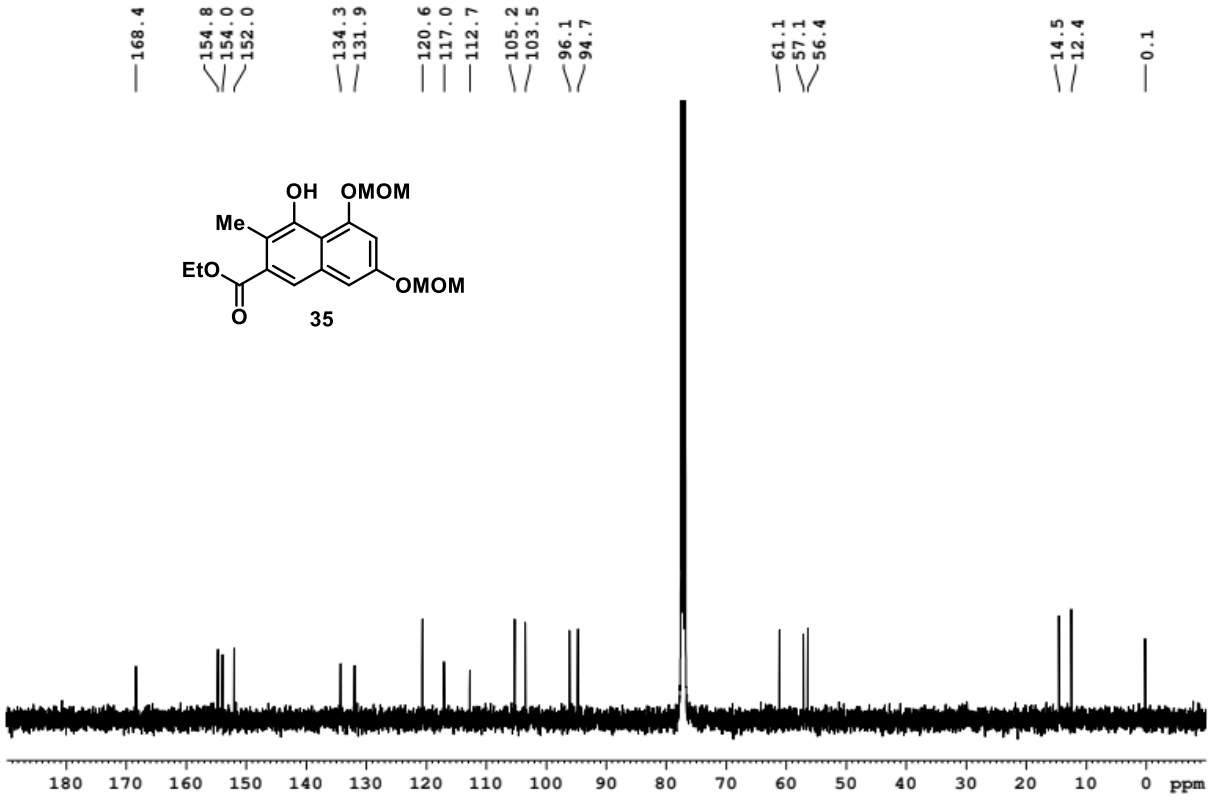
^{13}C NMR (100 MHz, $CDCl_3$)



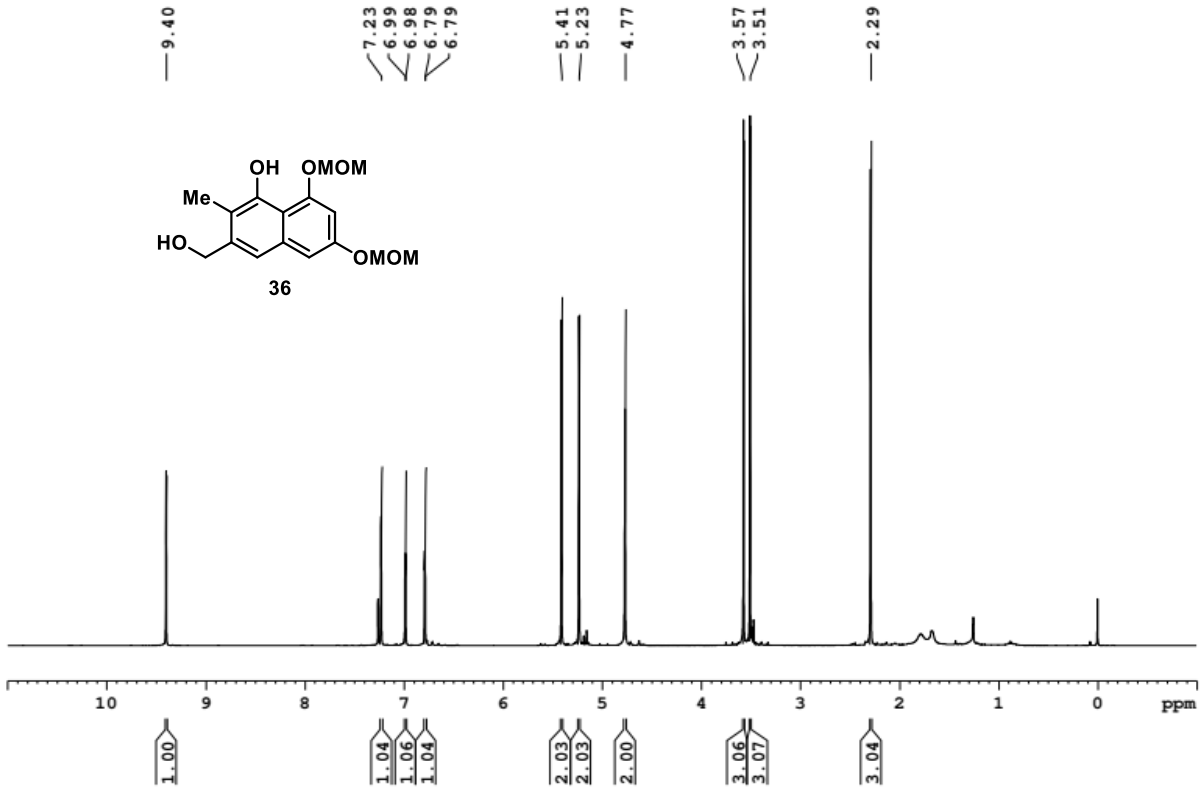
^{1}H NMR (400 MHz, $CDCl_3$)



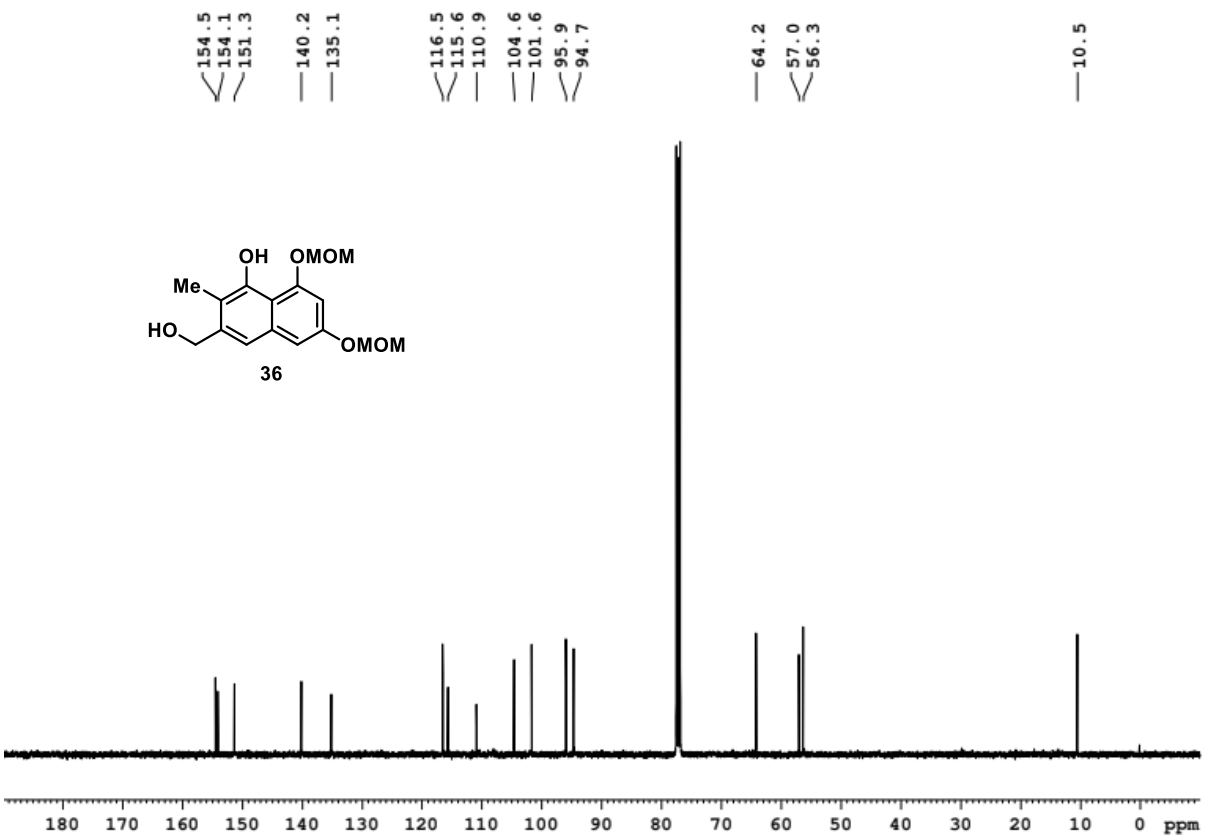
^{13}C NMR (100 MHz, $CDCl_3$)



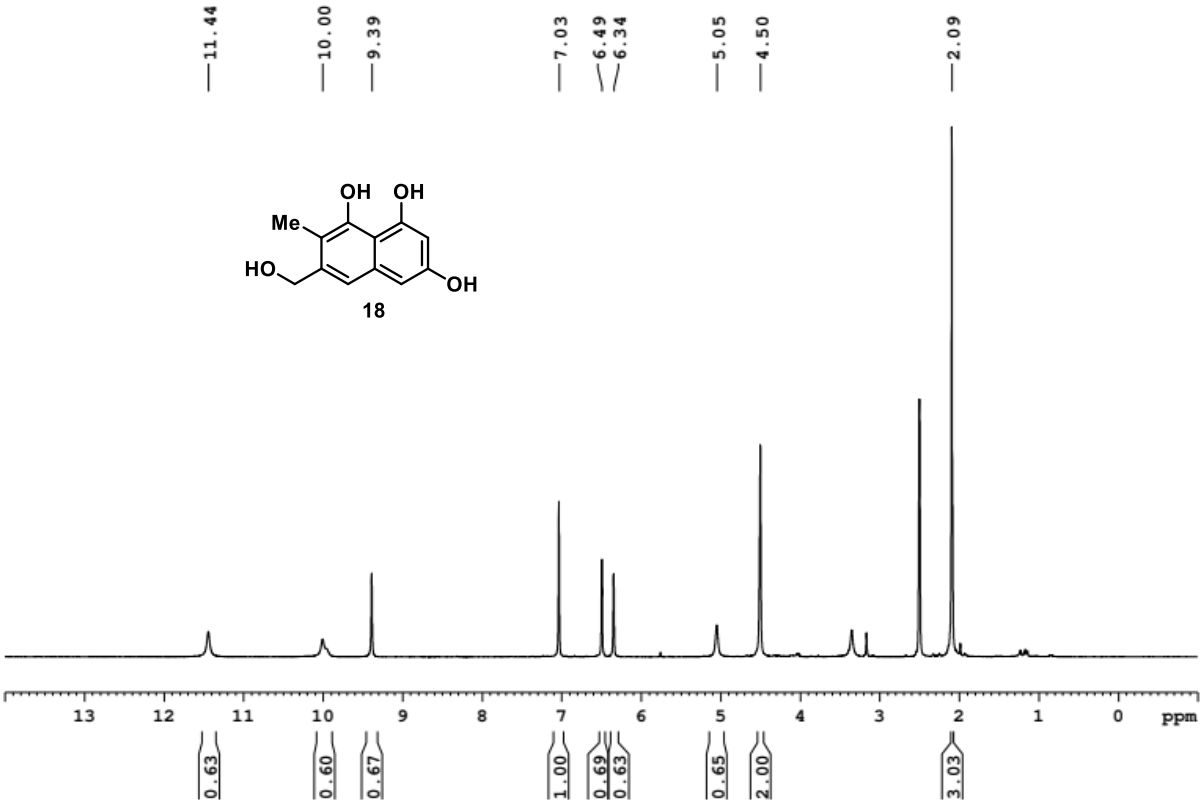
^{1}H NMR (400 MHz, $CDCl_3$)



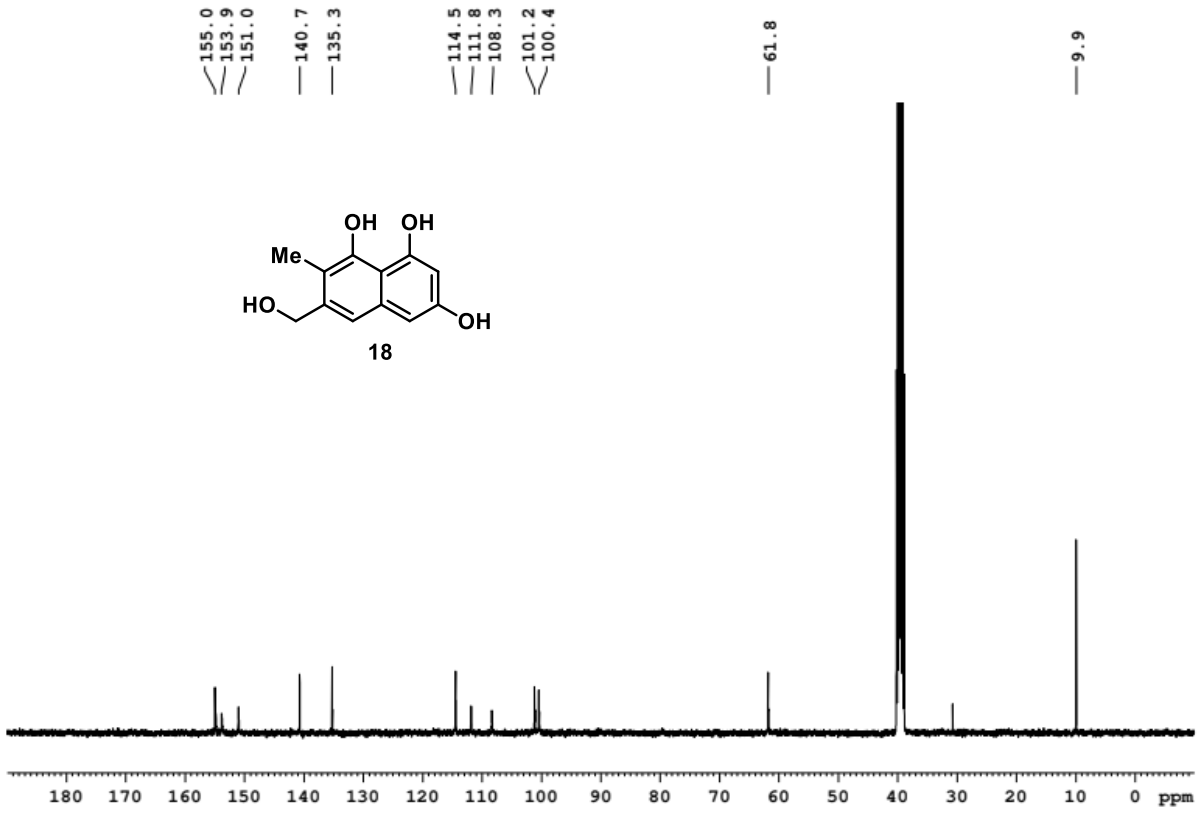
^{13}C NMR (100 MHz, $CDCl_3$)



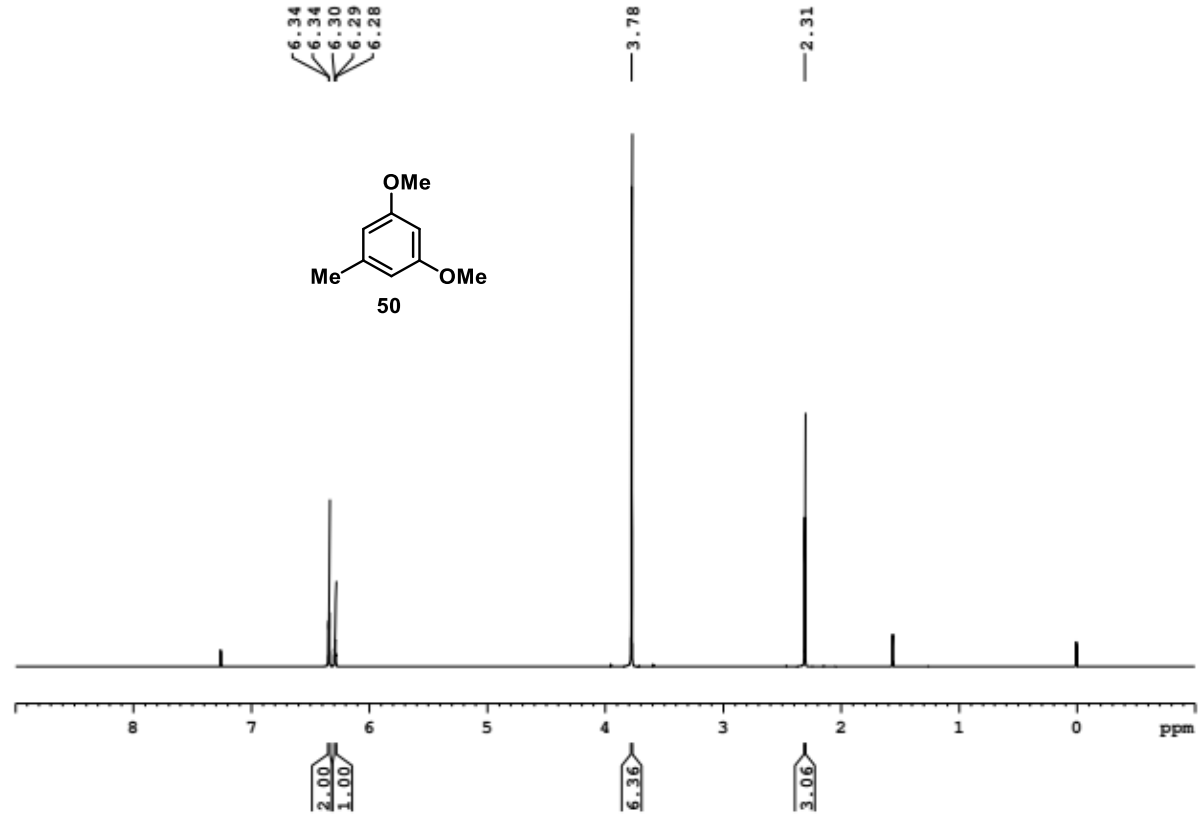
^{1}H NMR (400 MHz, acetone-d_6)



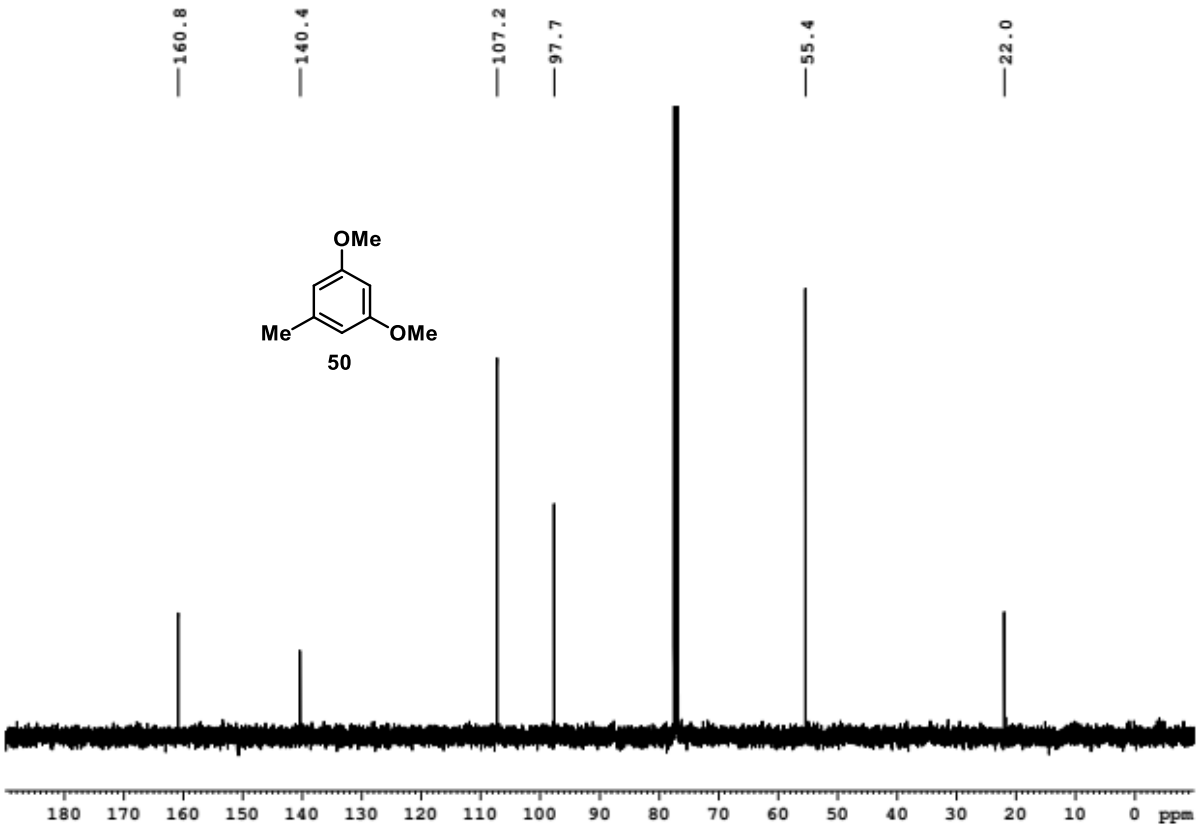
^{13}C NMR (100 MHz, acetone-d_6)



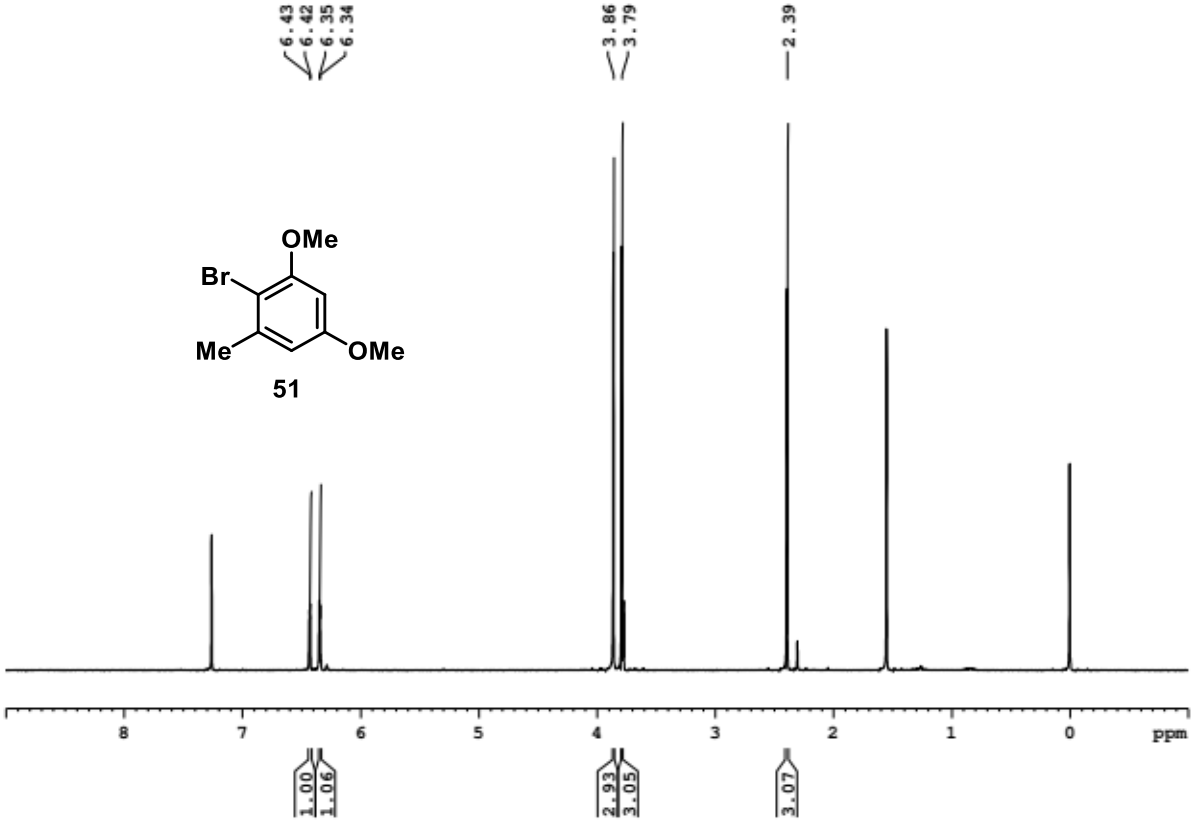
^{1}H NMR (400 MHz, $CDCl_3$)



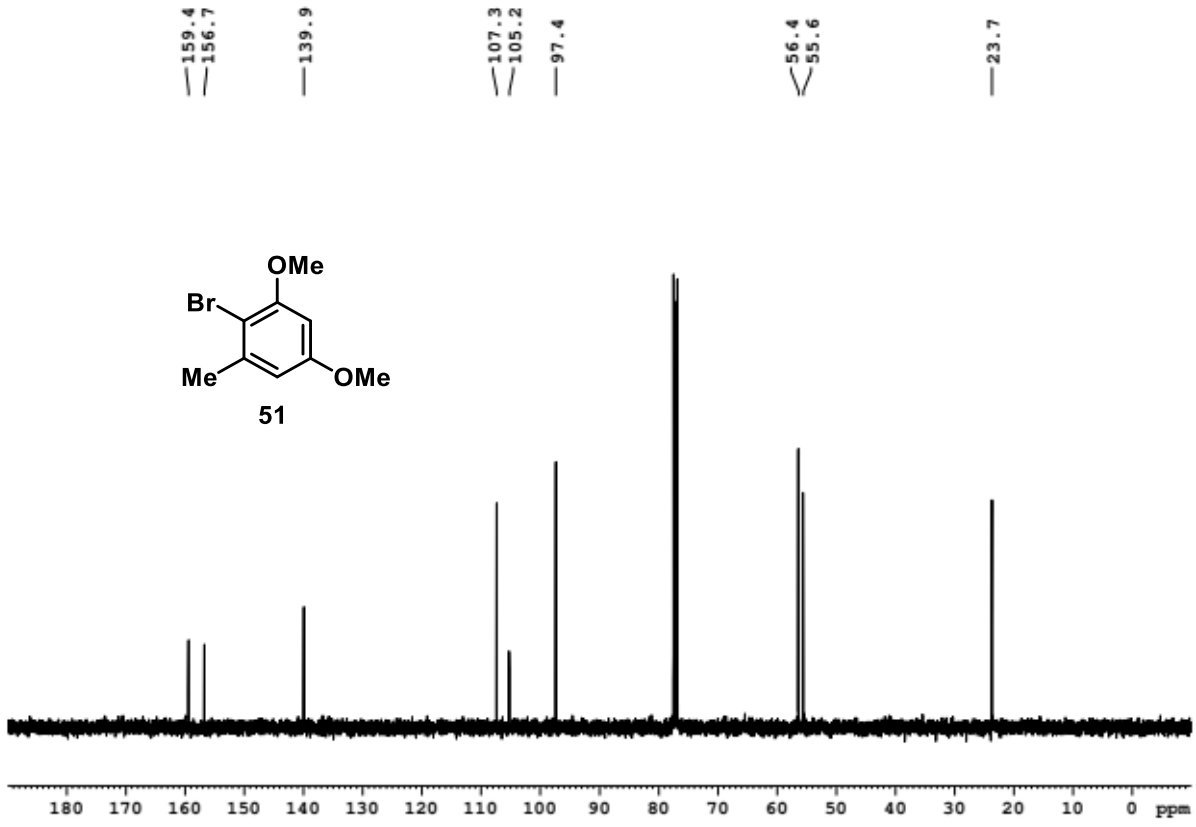
^{13}C NMR (100 MHz, $CDCl_3$)



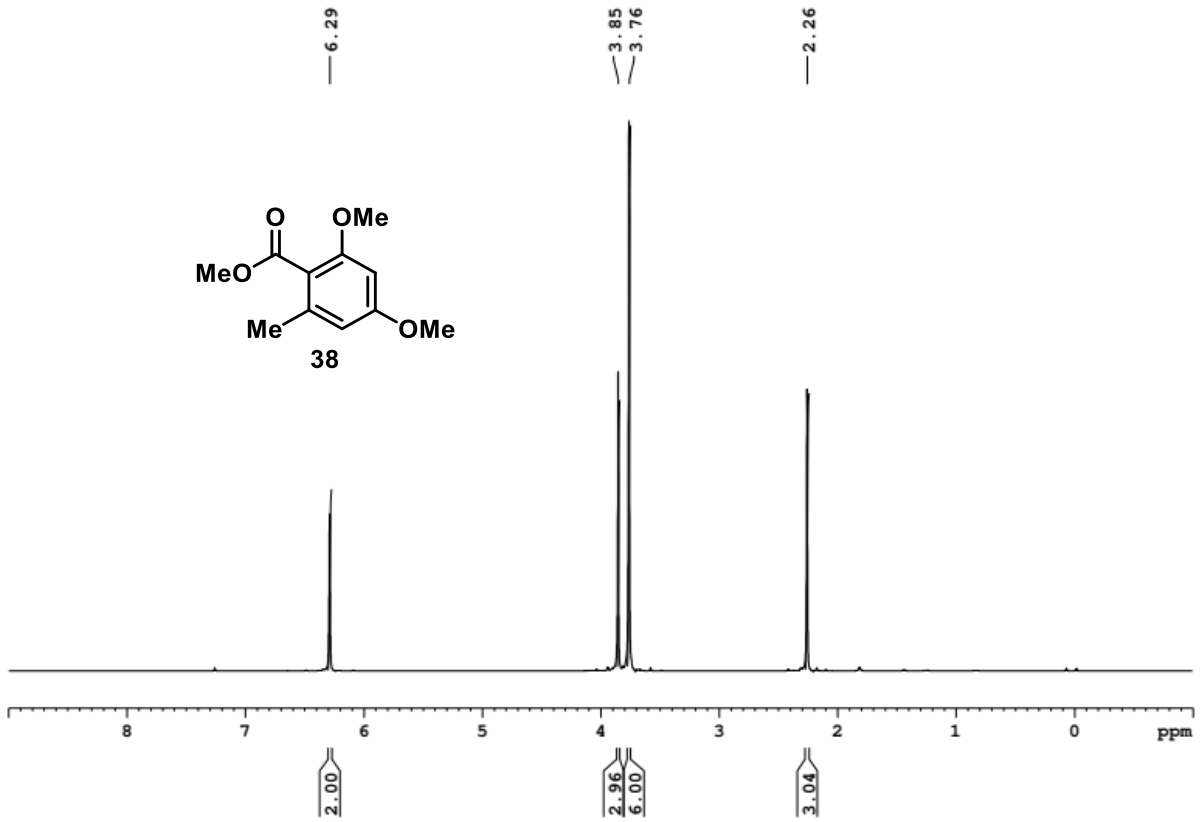
^{1}H NMR (400 MHz, $CDCl_3$)



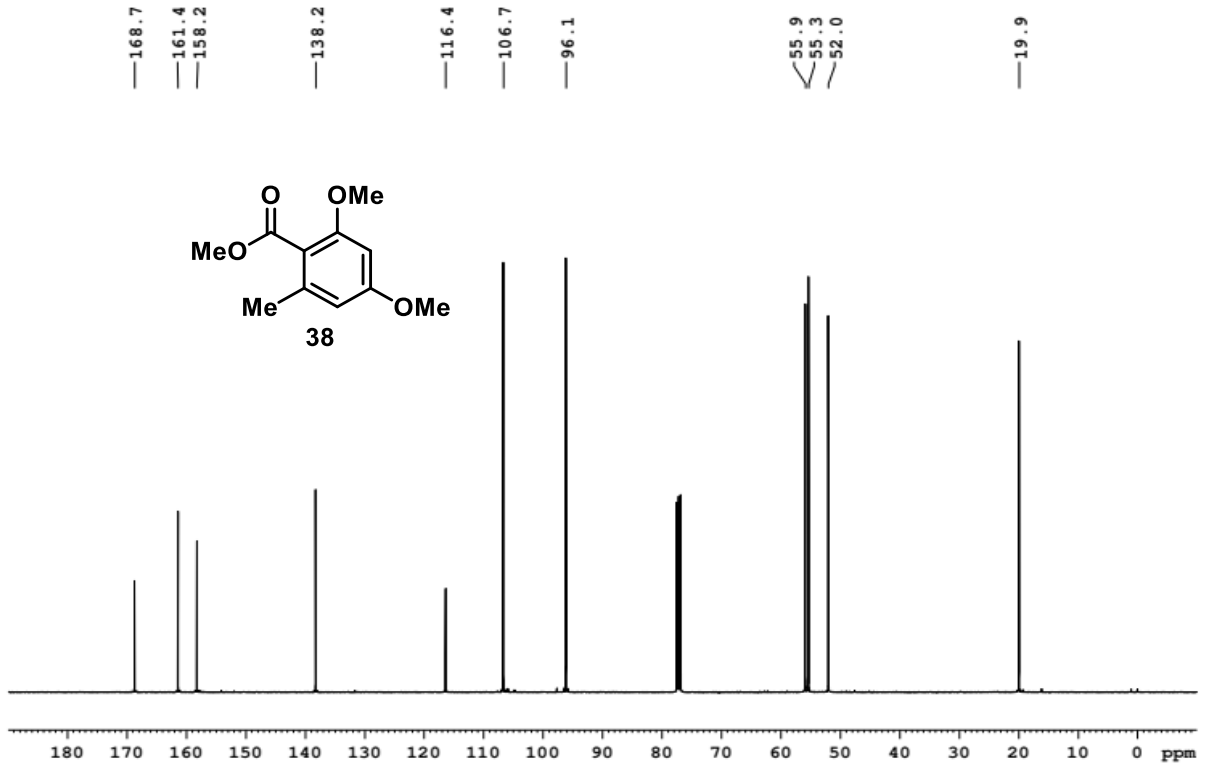
^{13}C NMR (100 MHz, $CDCl_3$)



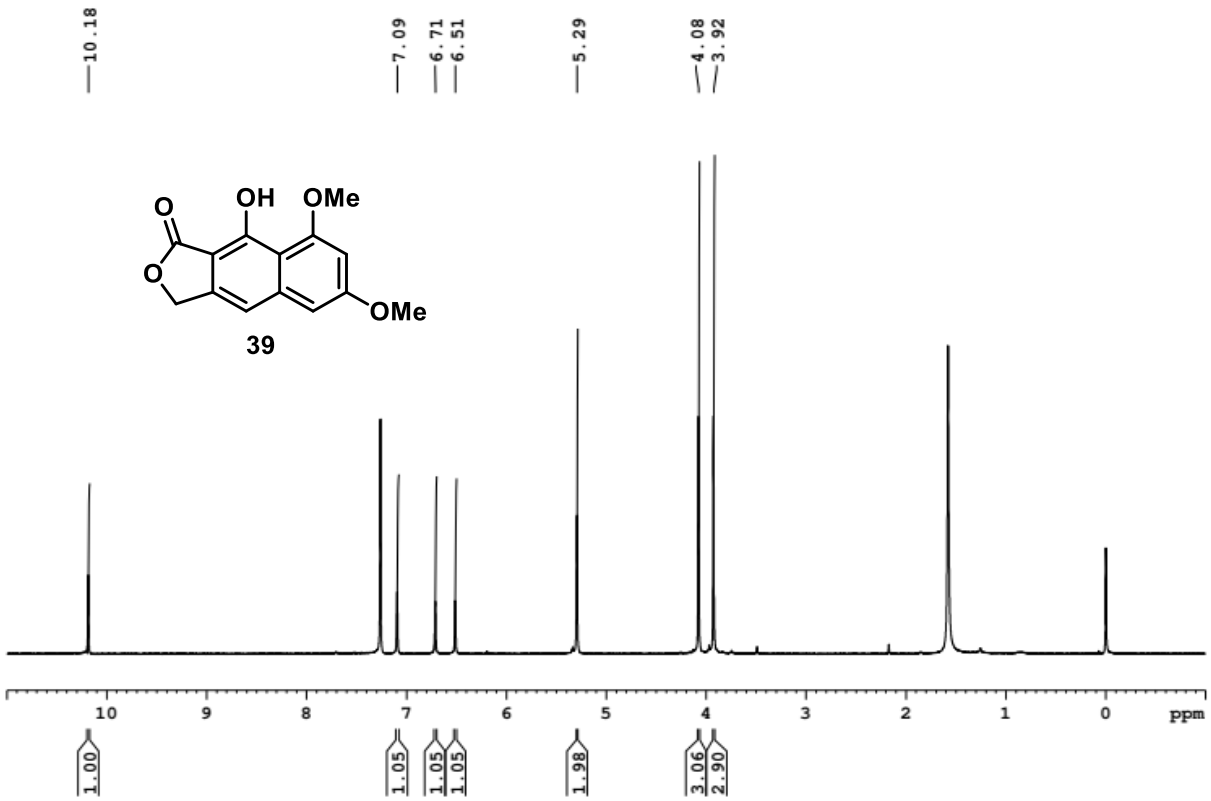
^{1}H NMR (400 MHz, $CDCl_3$)



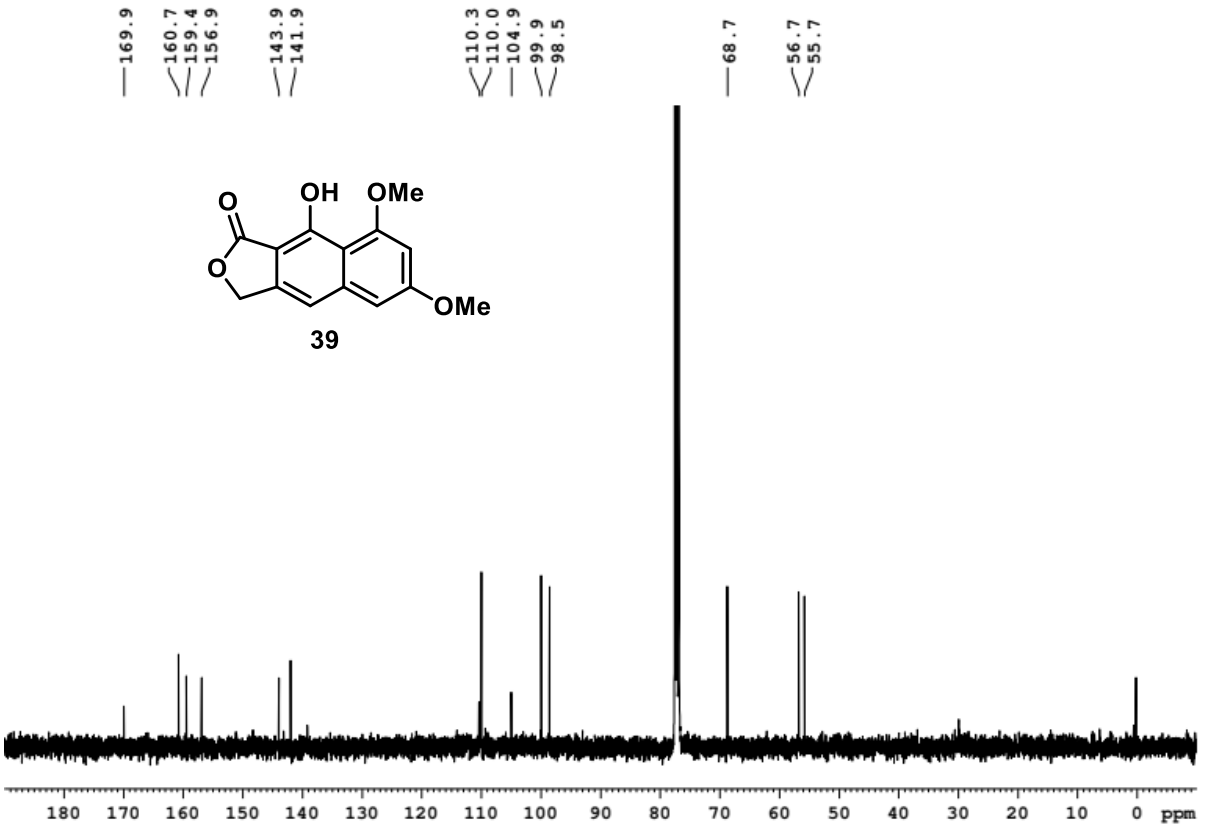
^{13}C NMR (100 MHz, $CDCl_3$)



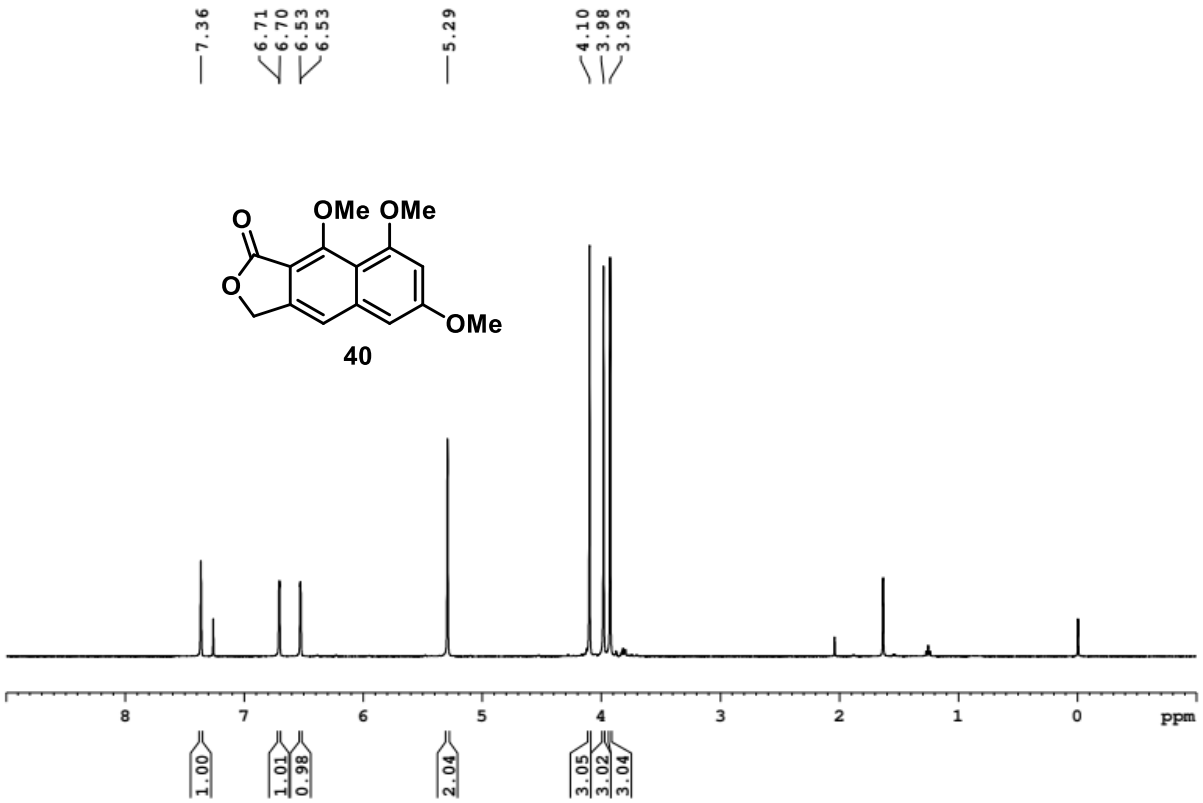
^{1}H NMR (400 MHz, $CDCl_3$)



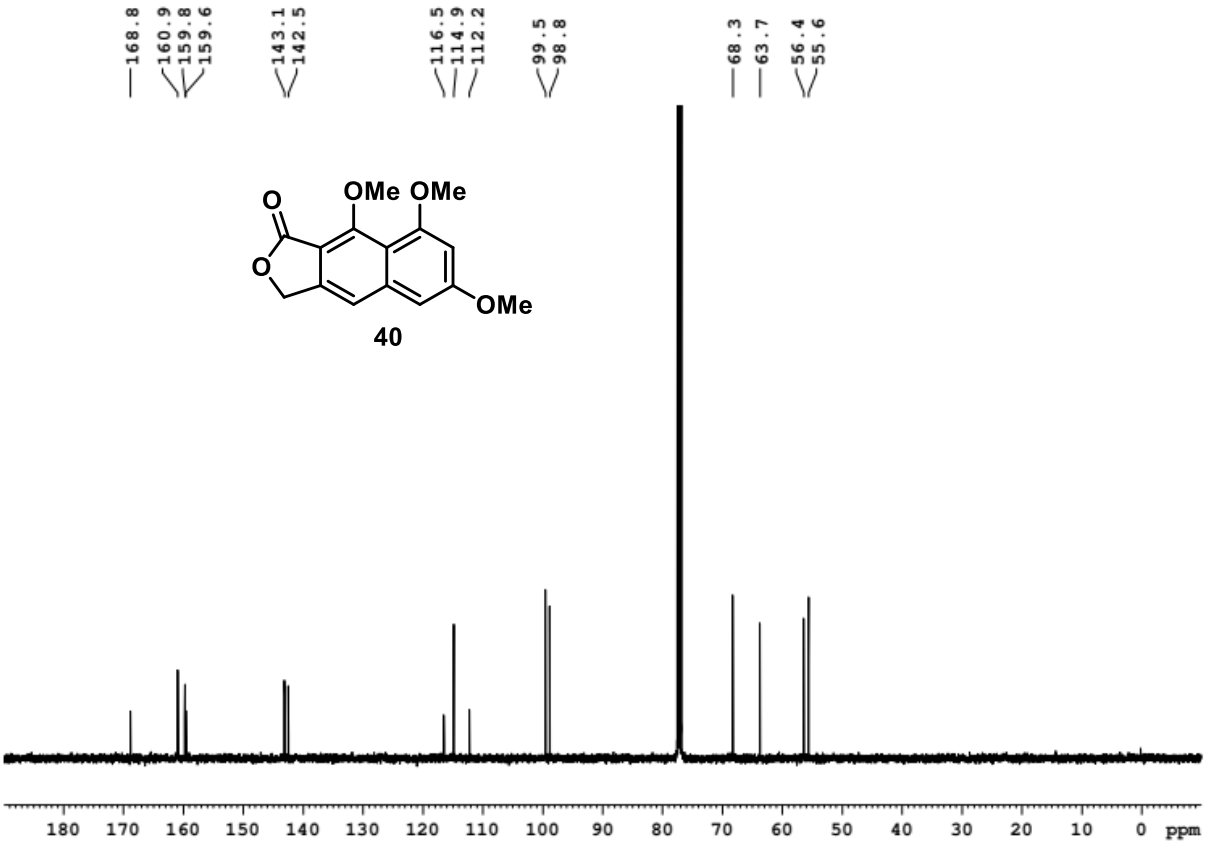
^{13}C NMR (100 MHz, $CDCl_3$)



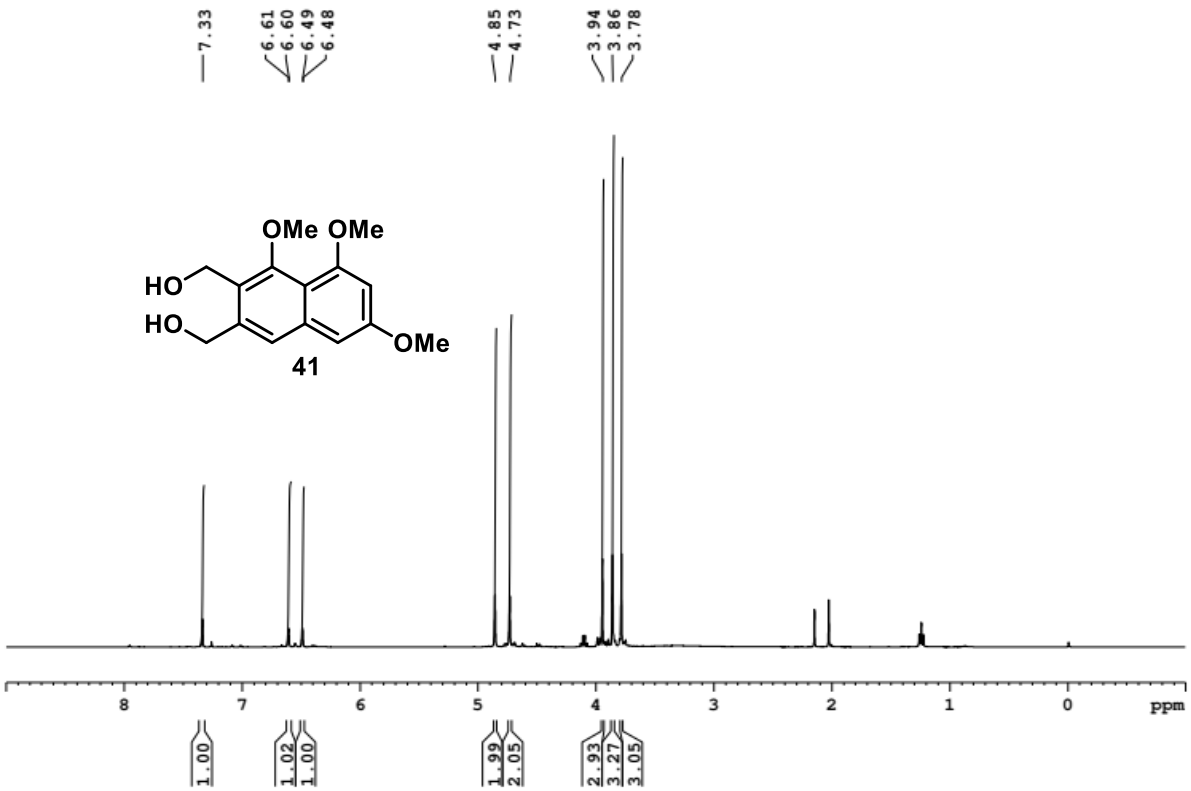
^{1}H NMR (400 MHz, $CDCl_3$)



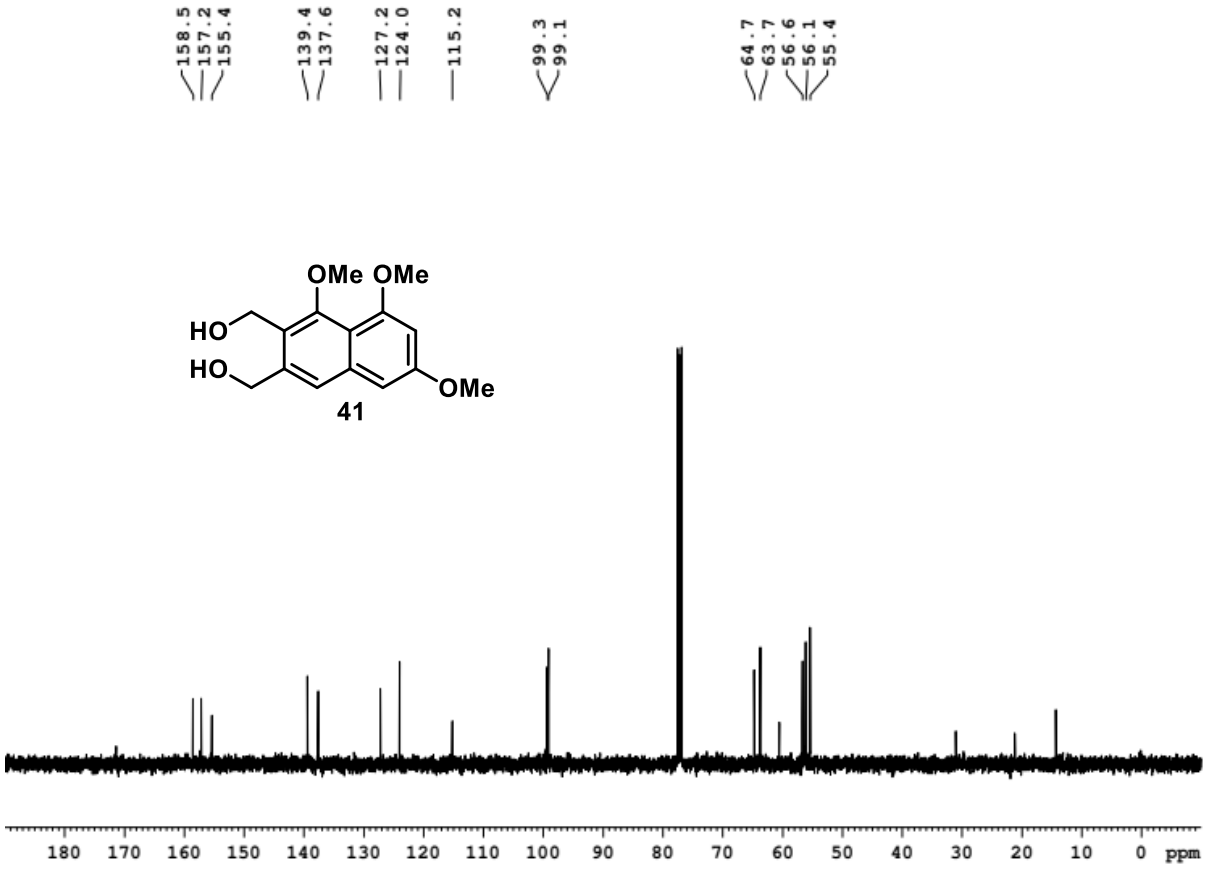
^{13}C NMR (100 MHz, $CDCl_3$)



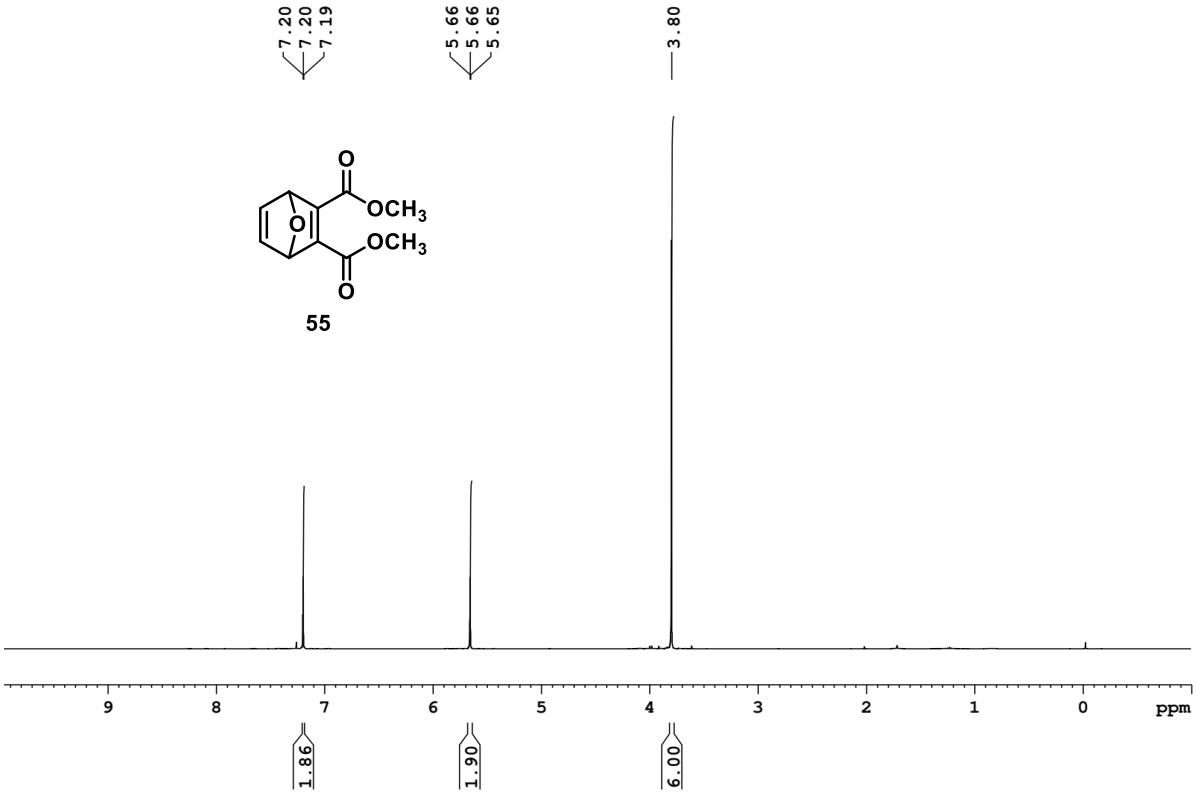
^{1}H NMR (400 MHz, $CDCl_3$)



^{13}C NMR (100 MHz, $CDCl_3$)

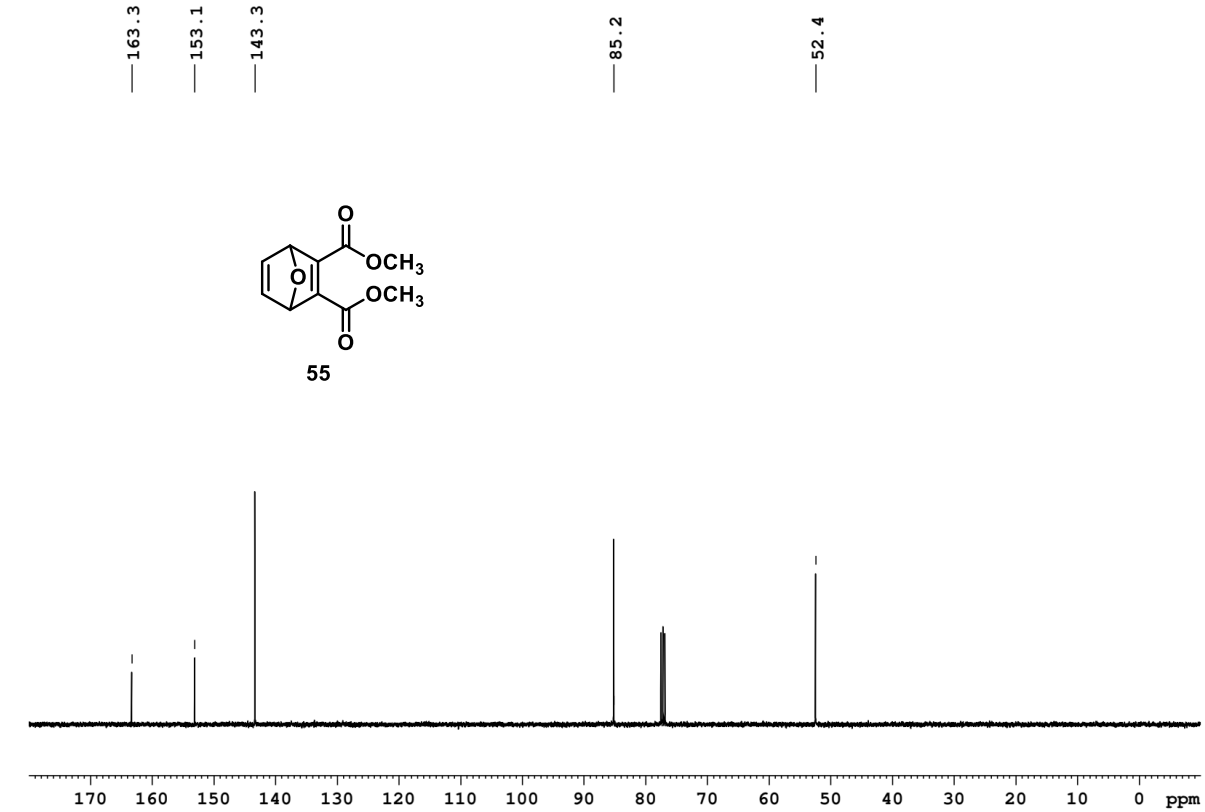


^{1}H NMR (400 MHz, $CDCl_3$)



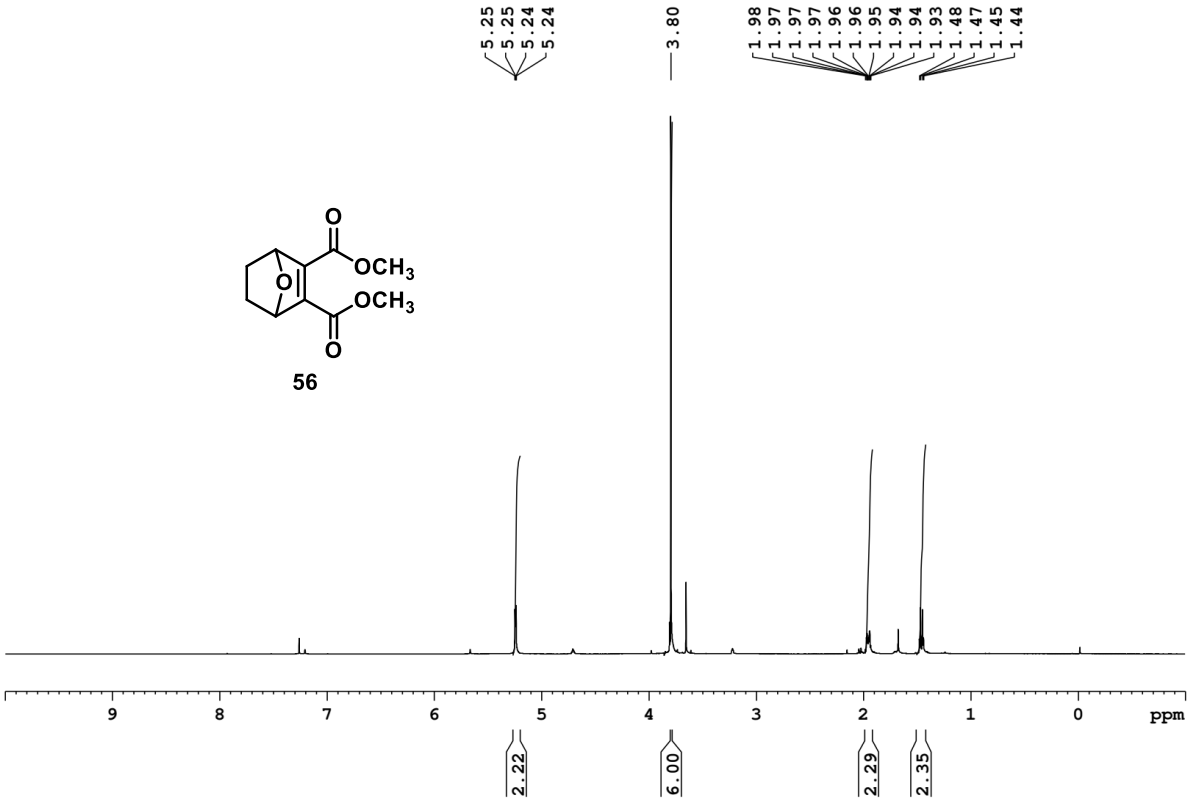
55

^{13}C NMR (100 MHz, $CDCl_3$)



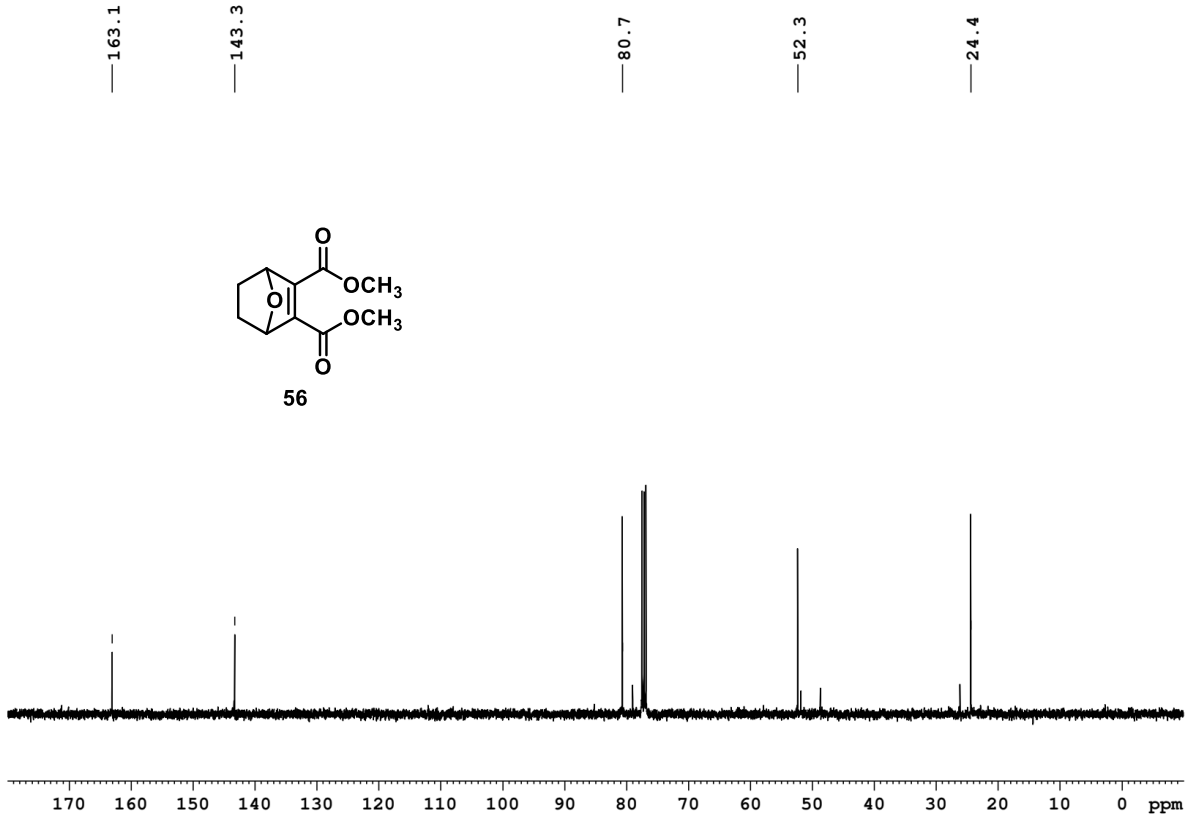
55

^{1}H NMR (400 MHz, $CDCl_3$)



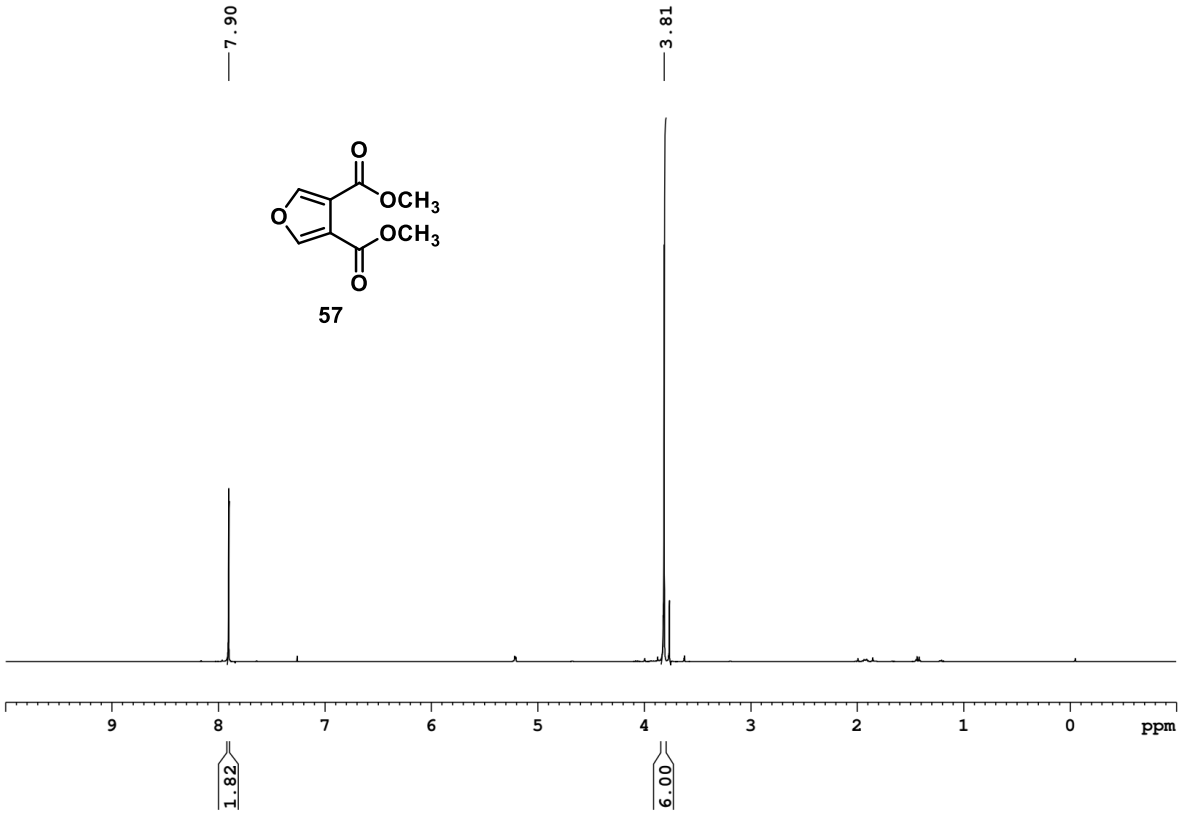
56

^{13}C NMR (100 MHz, $CDCl_3$)

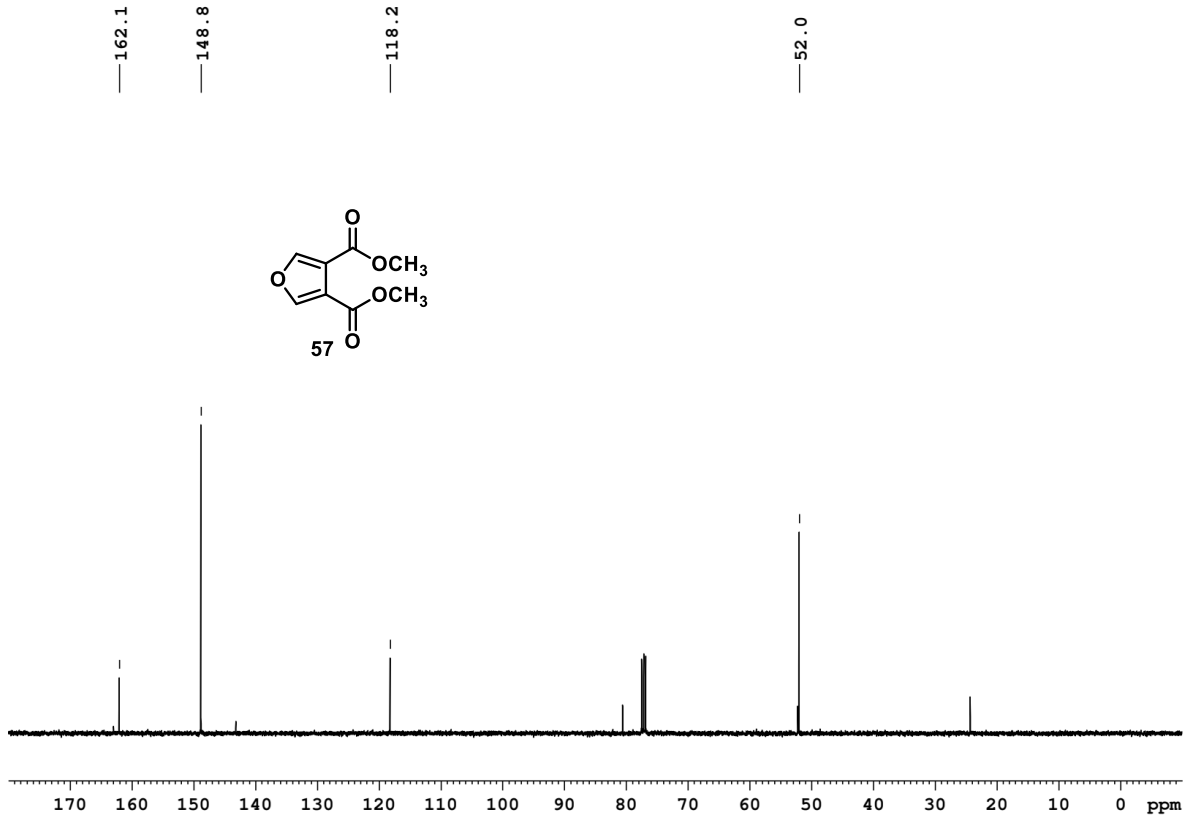


56

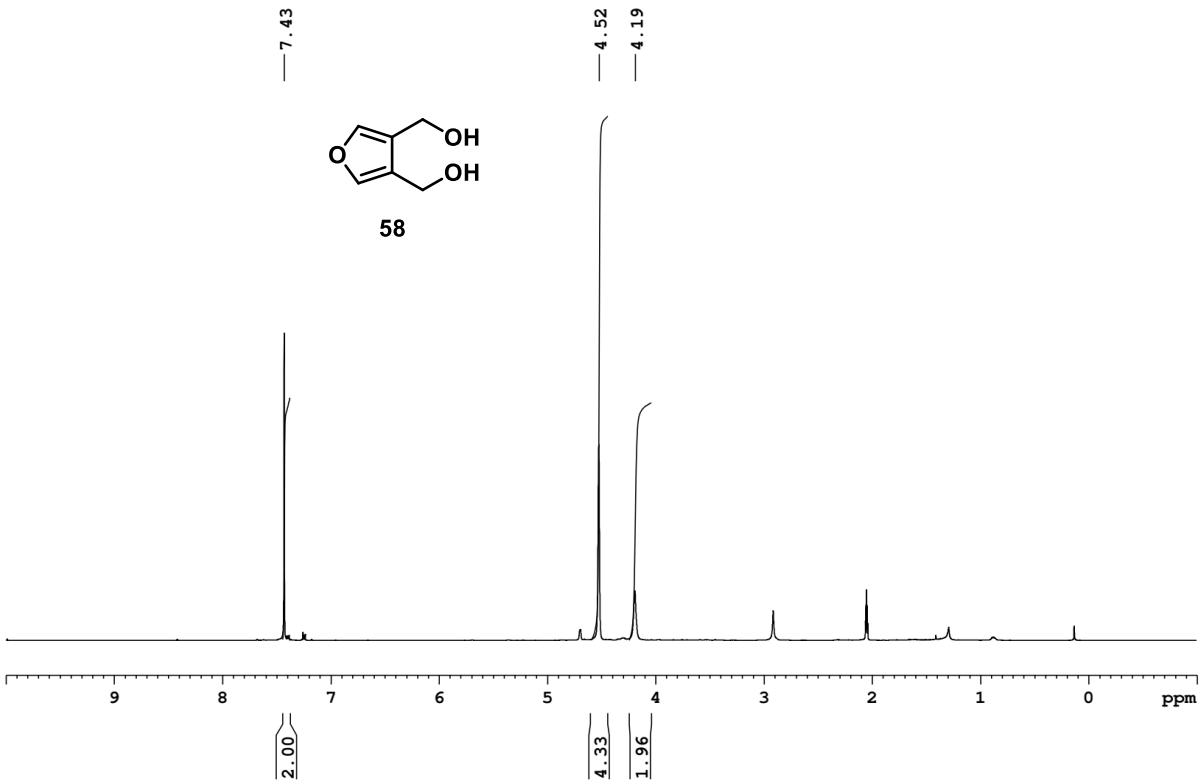
^{1}H NMR (400 MHz, $CDCl_3$)



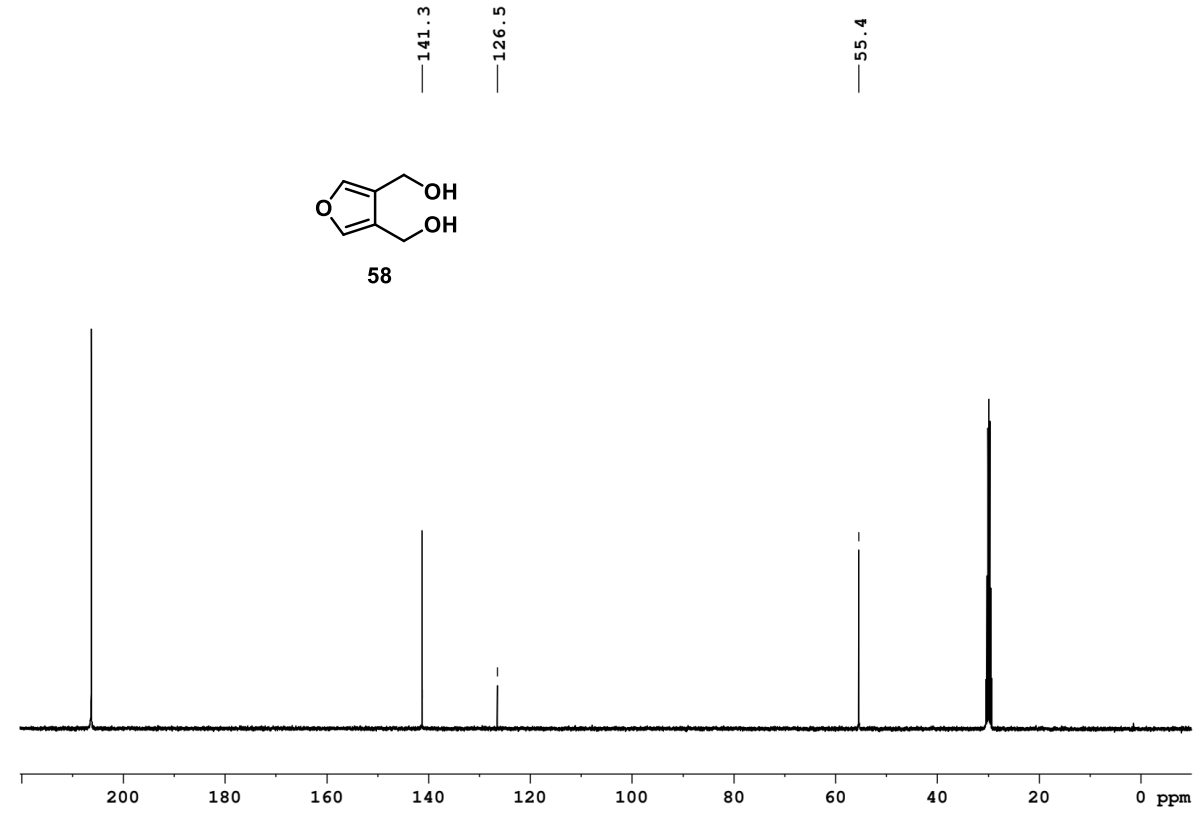
^{13}C NMR (100 MHz, $CDCl_3$)



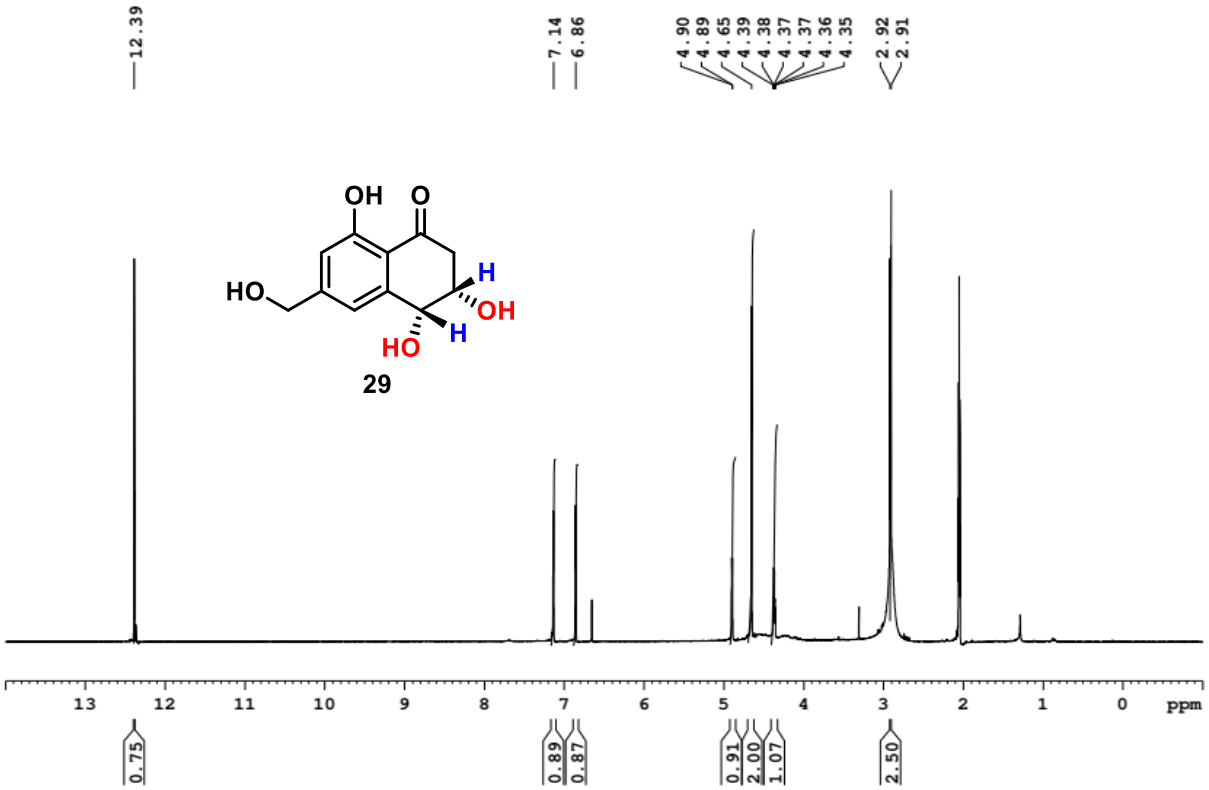
^{1}H NMR (400 MHz, acetone-d_6)



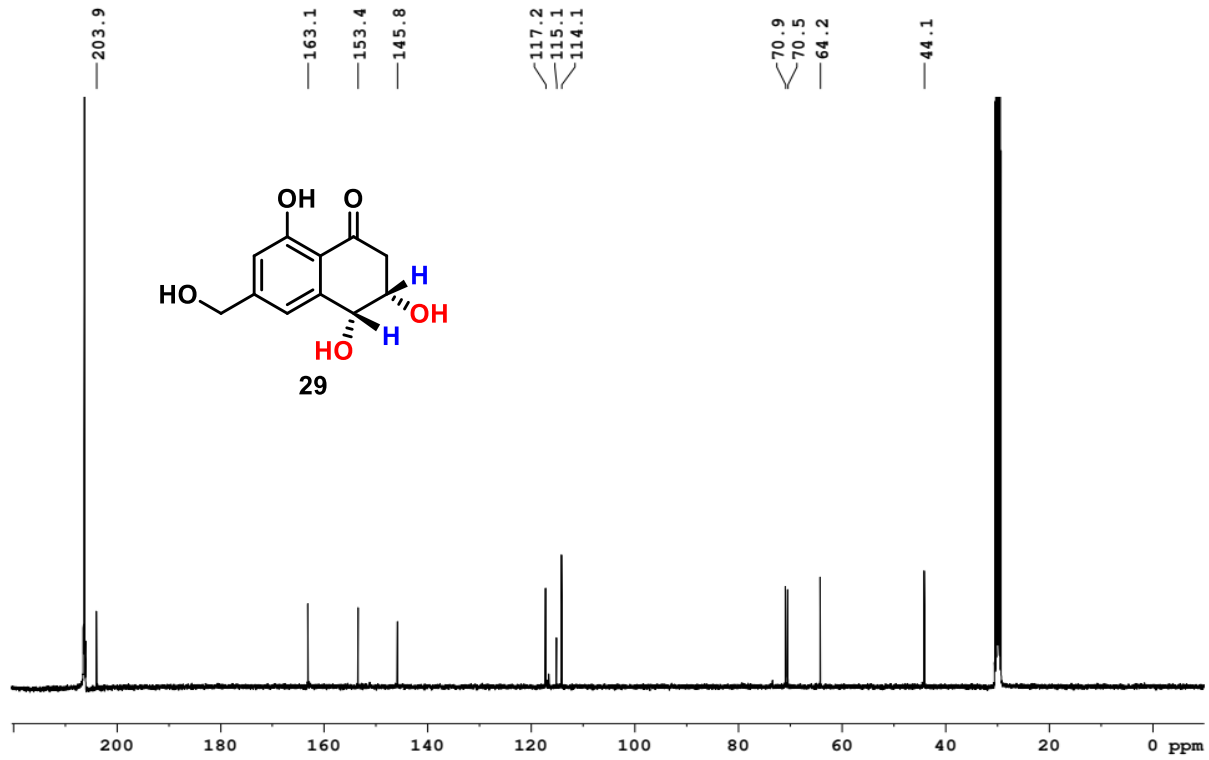
^{13}C NMR (100 MHz, acetone-d_6)



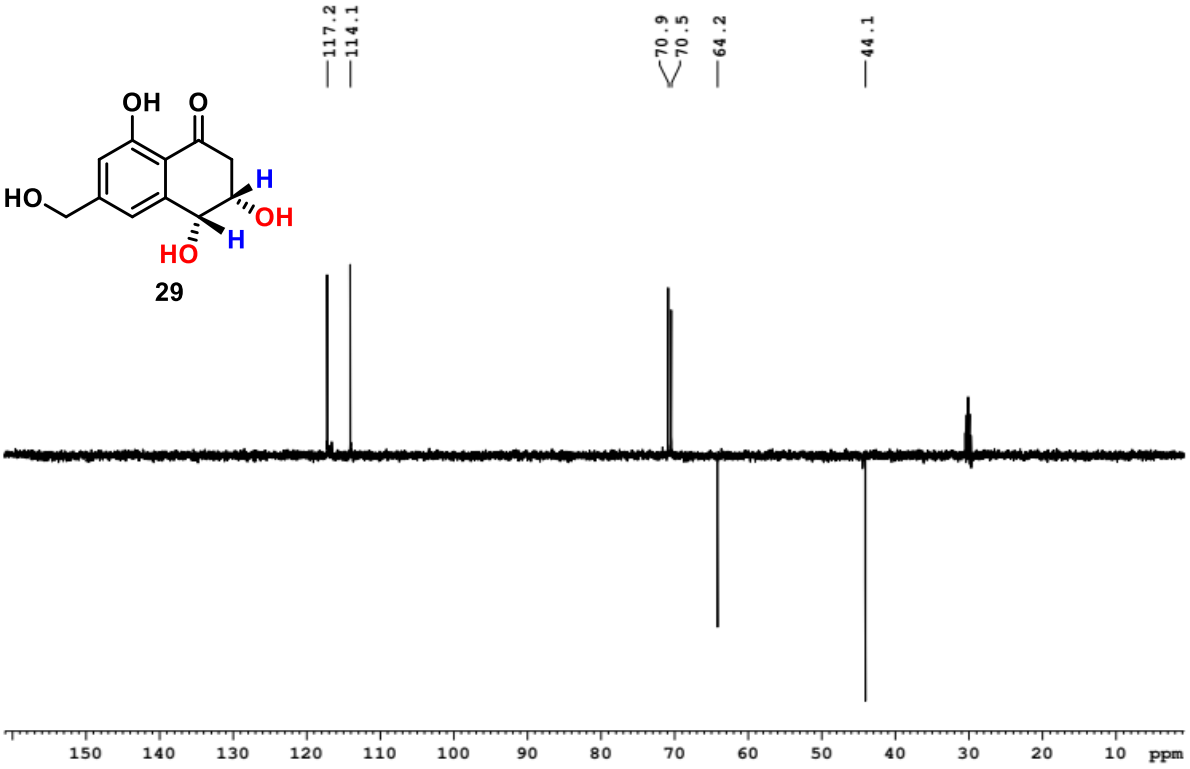
^{1}H NMR (400 MHz, acetone-d_6)



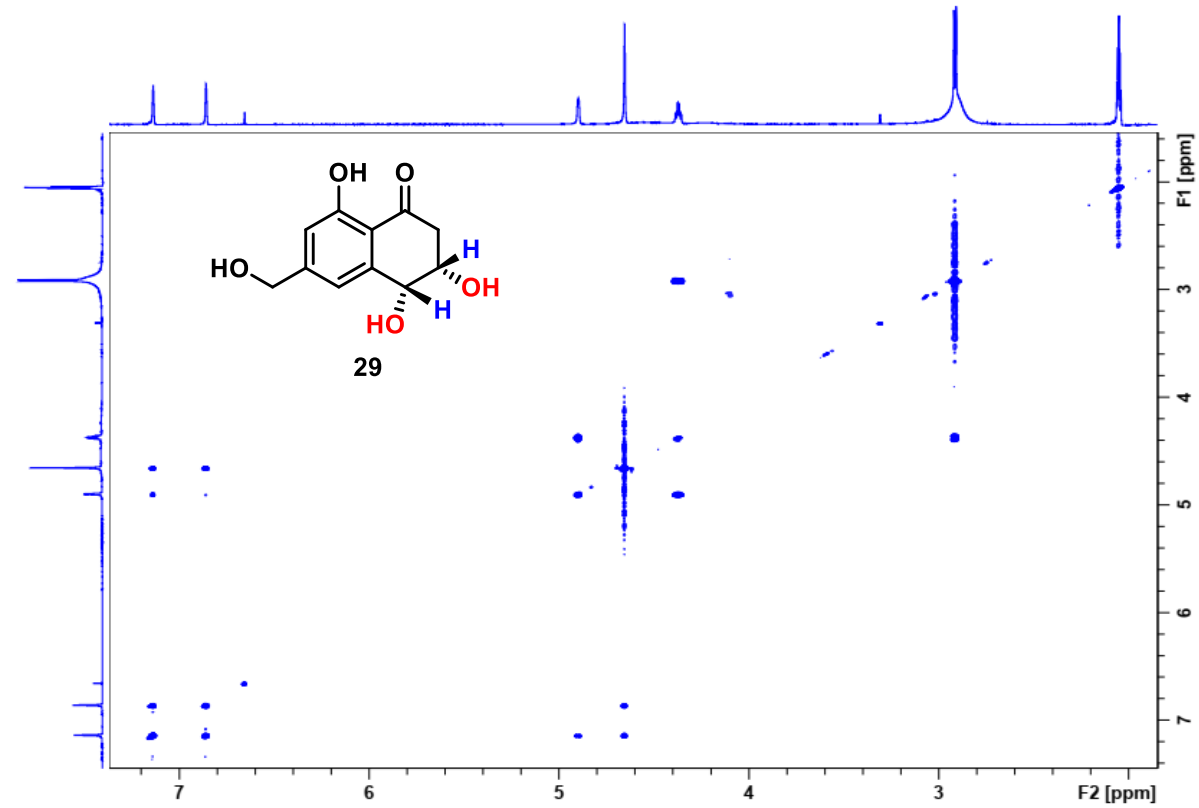
^{13}C NMR (100 MHz, acetone-d_6)



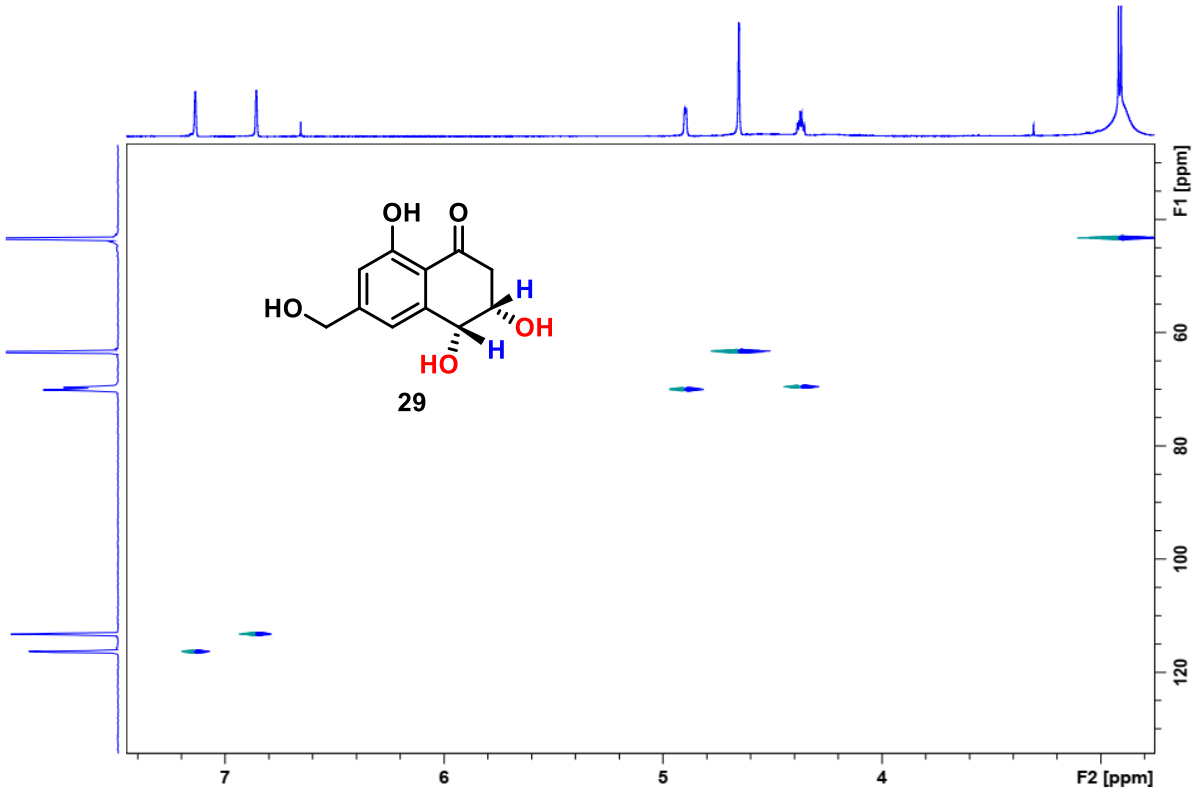
DEPT-135 NMR (100 MHz, acetone-*d*$_6$)



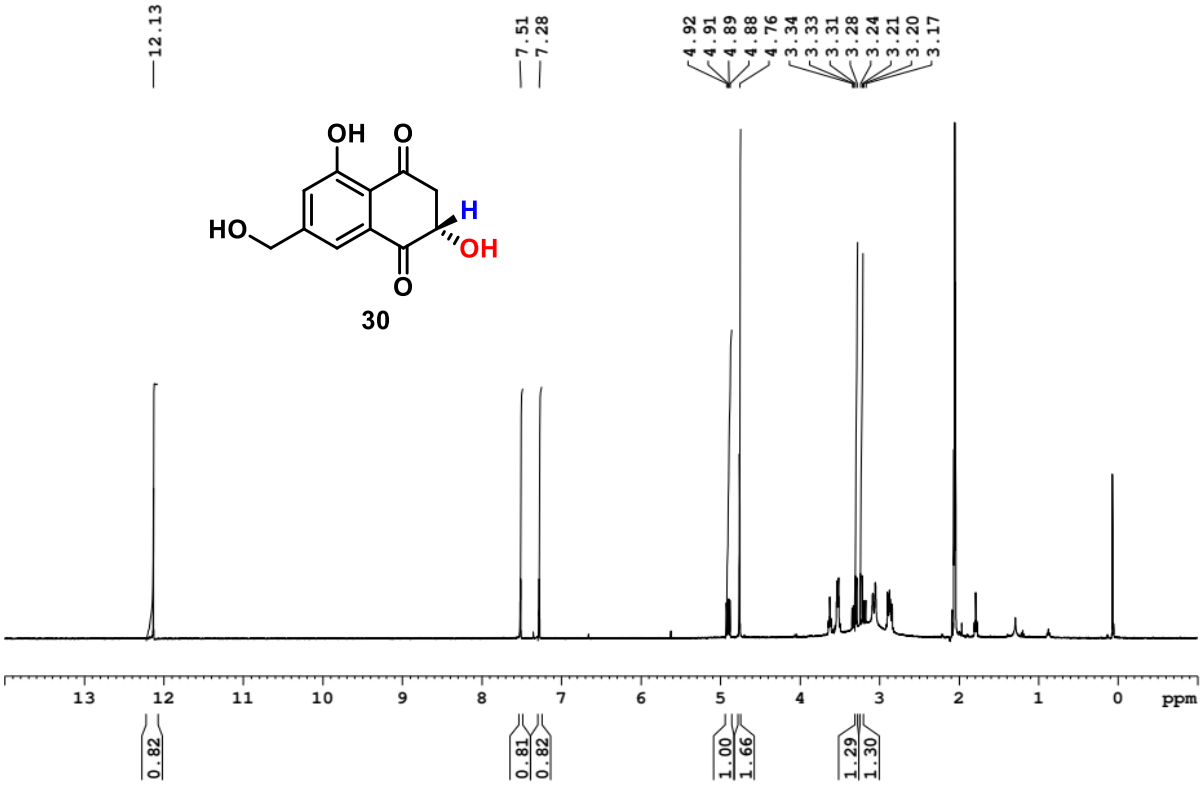
^{1}H-^{1}H COSY (400 MHz, acetone-*d*$_6$)



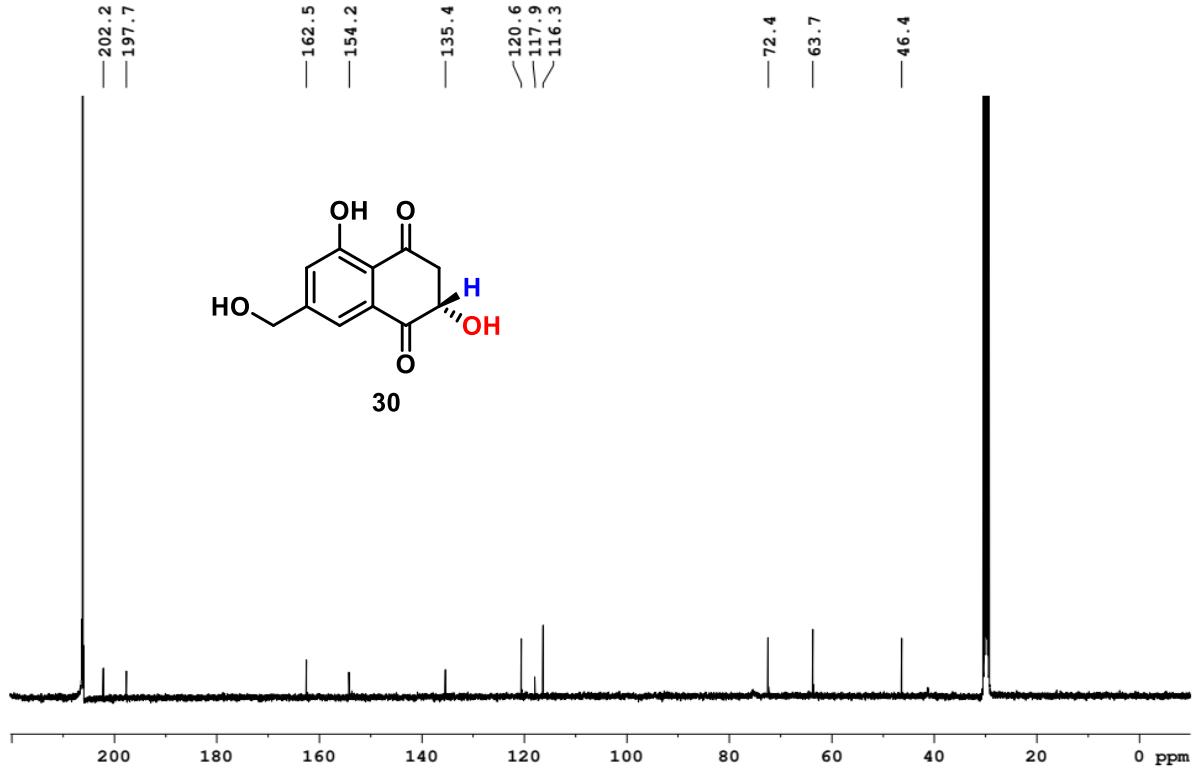
^{1}H-^{13}C HSQC (400 MHz, acetone-d_6)



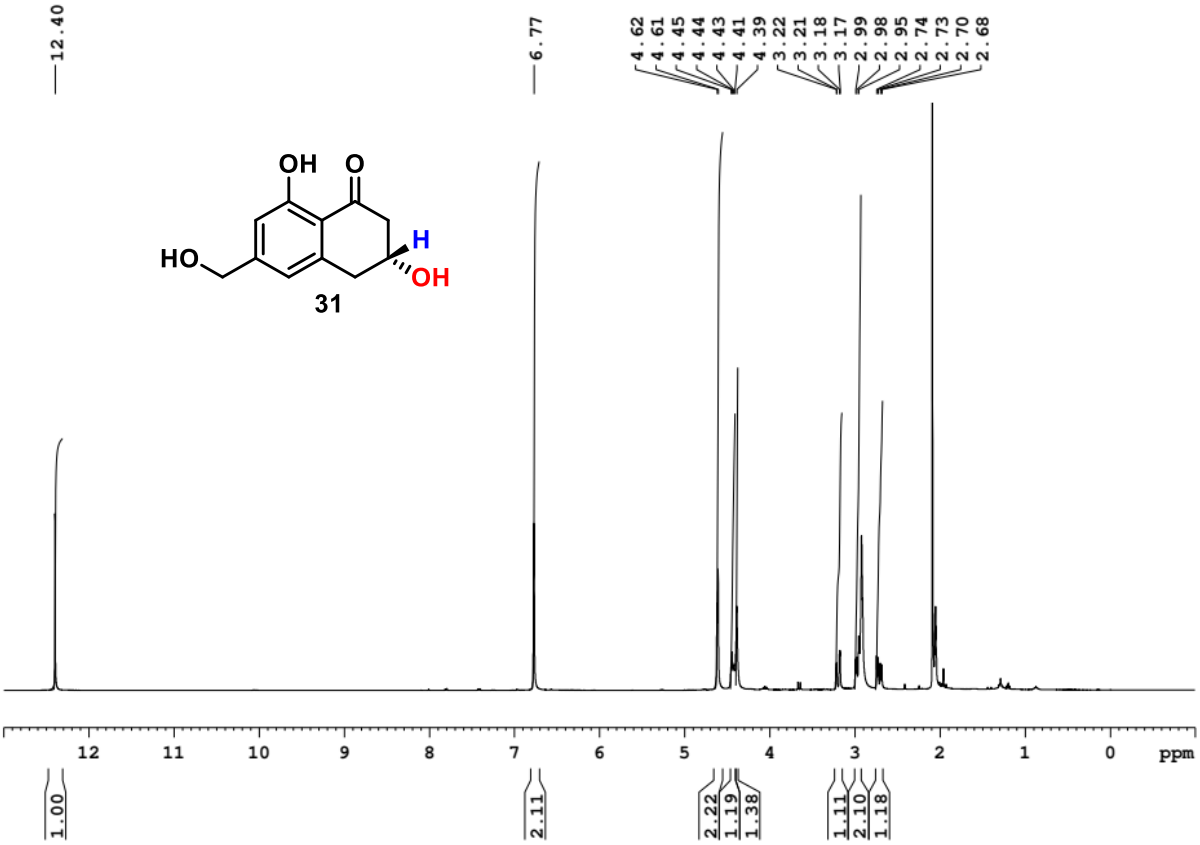
^{1}H NMR (400 MHz, acetone-d_6)



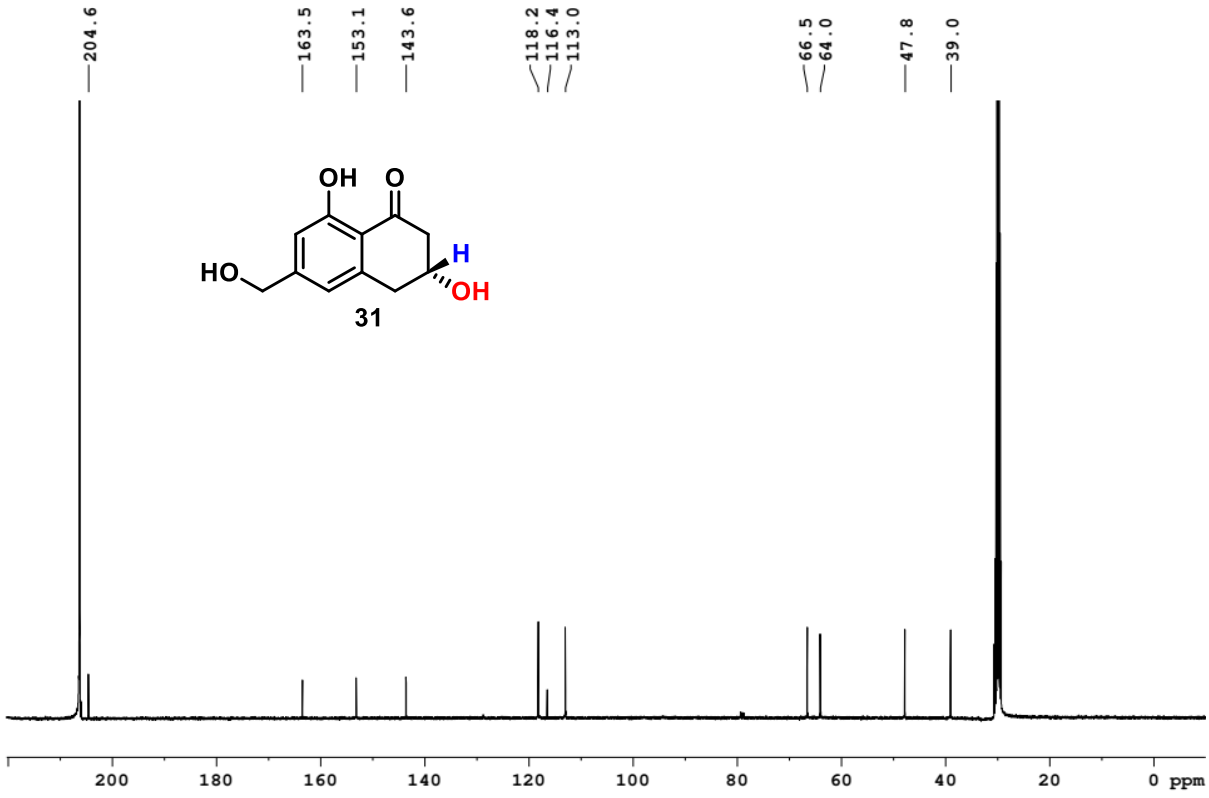
^{13}C NMR (100 MHz, acetone-d_6)



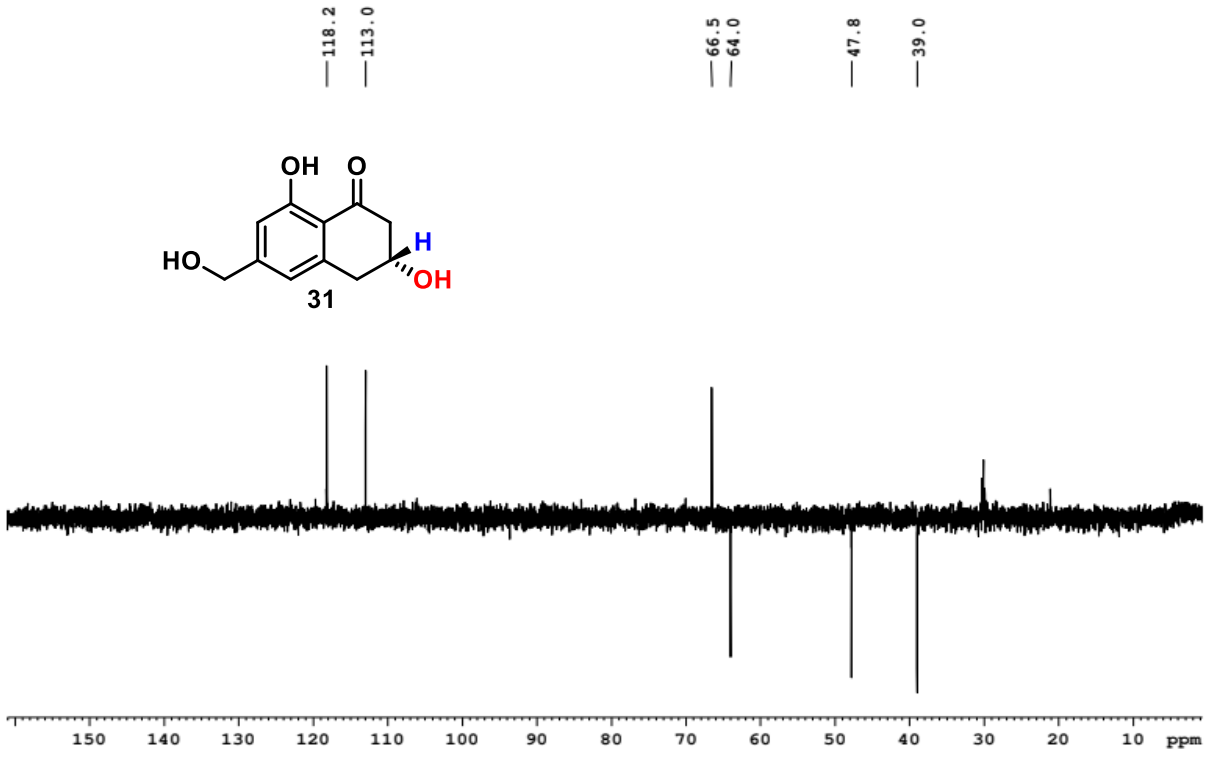
^{1}H NMR (400 MHz, acetone-d_6)



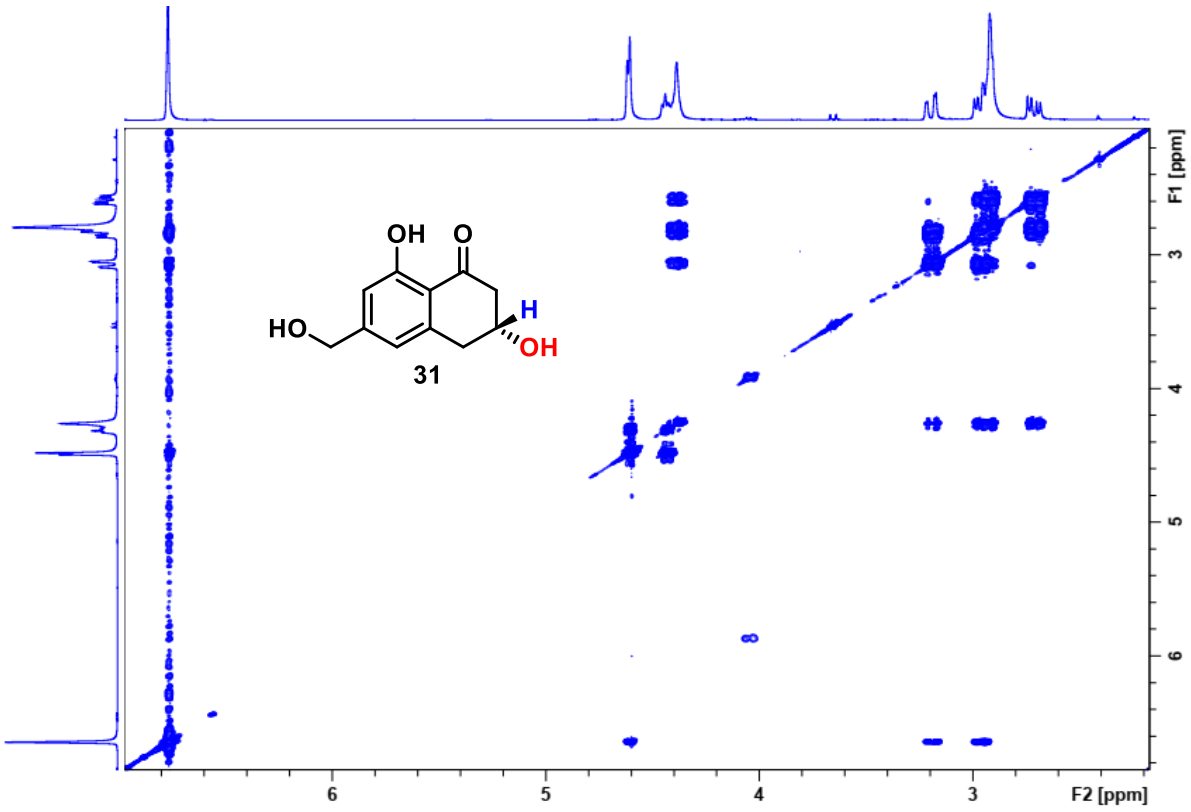
^{13}C NMR (100 MHz, acetone-*d*$_6$)



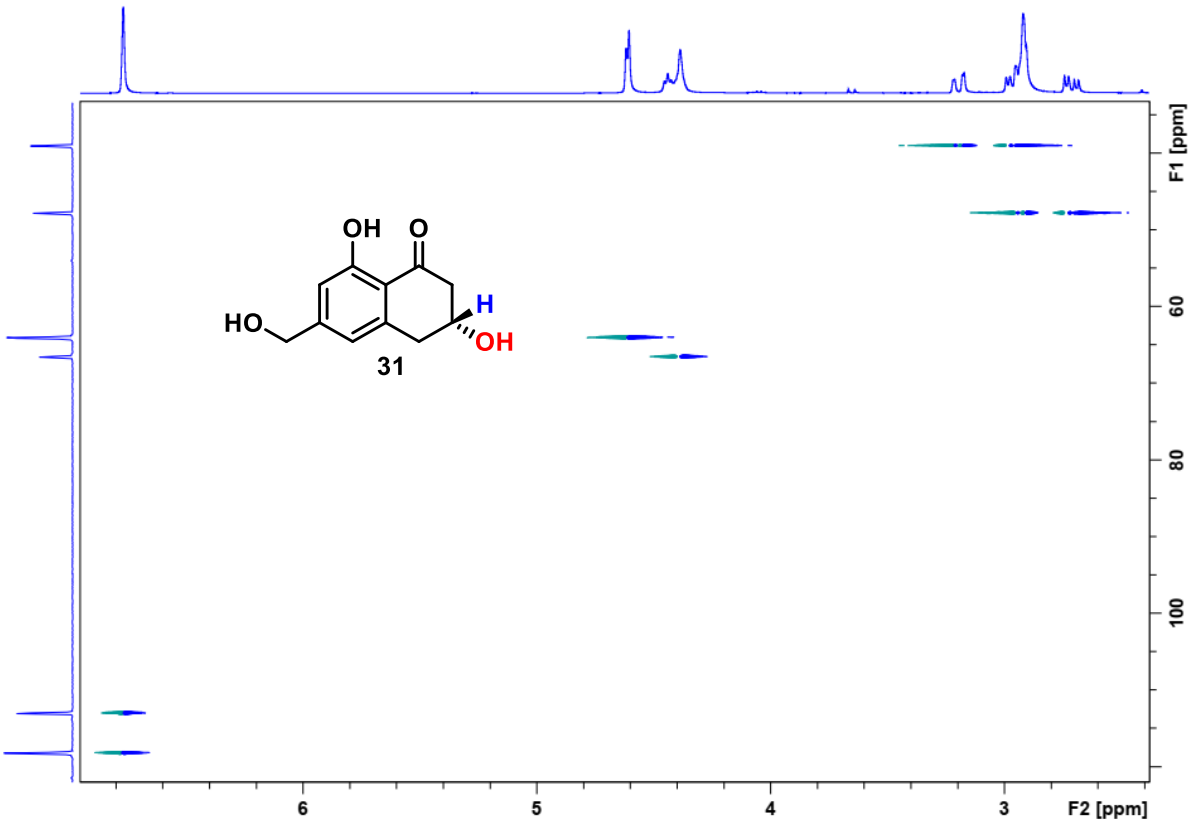
DEPT-135 NMR (100 MHz, acetone-*d*$_6$)



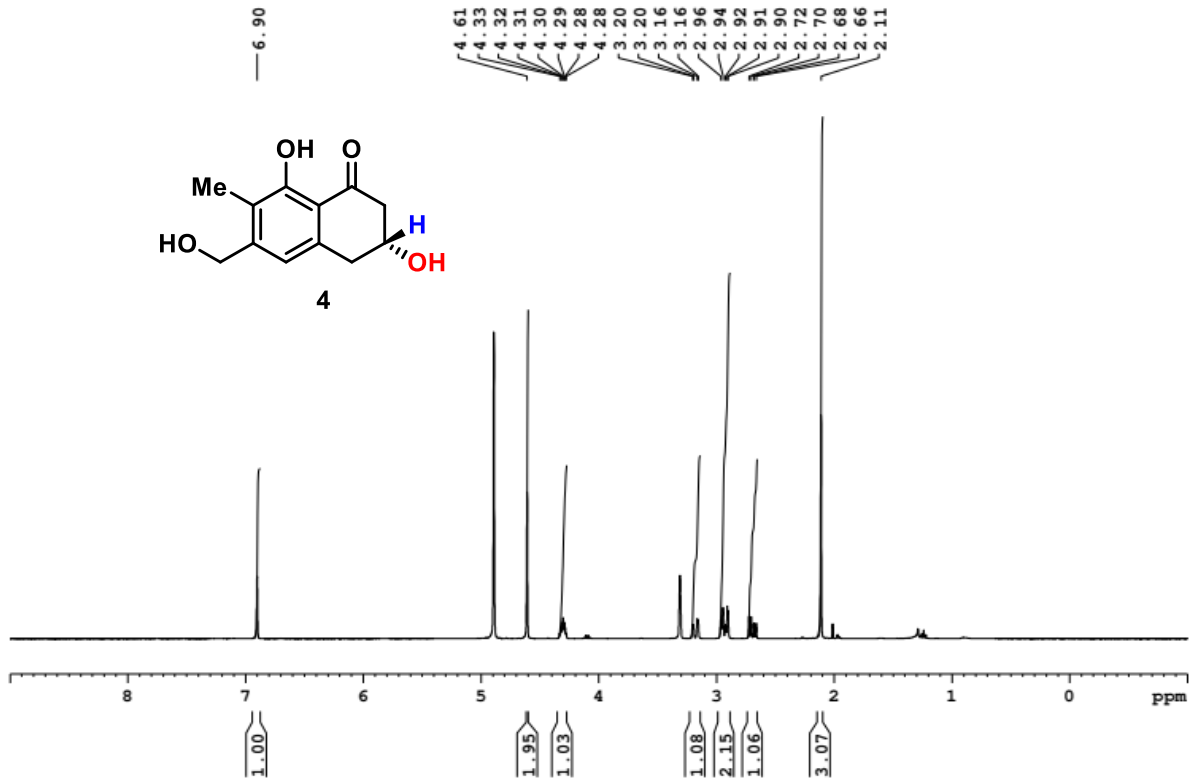
^{1}H-^{1}H COSY (400 MHz, acetone-d_6)



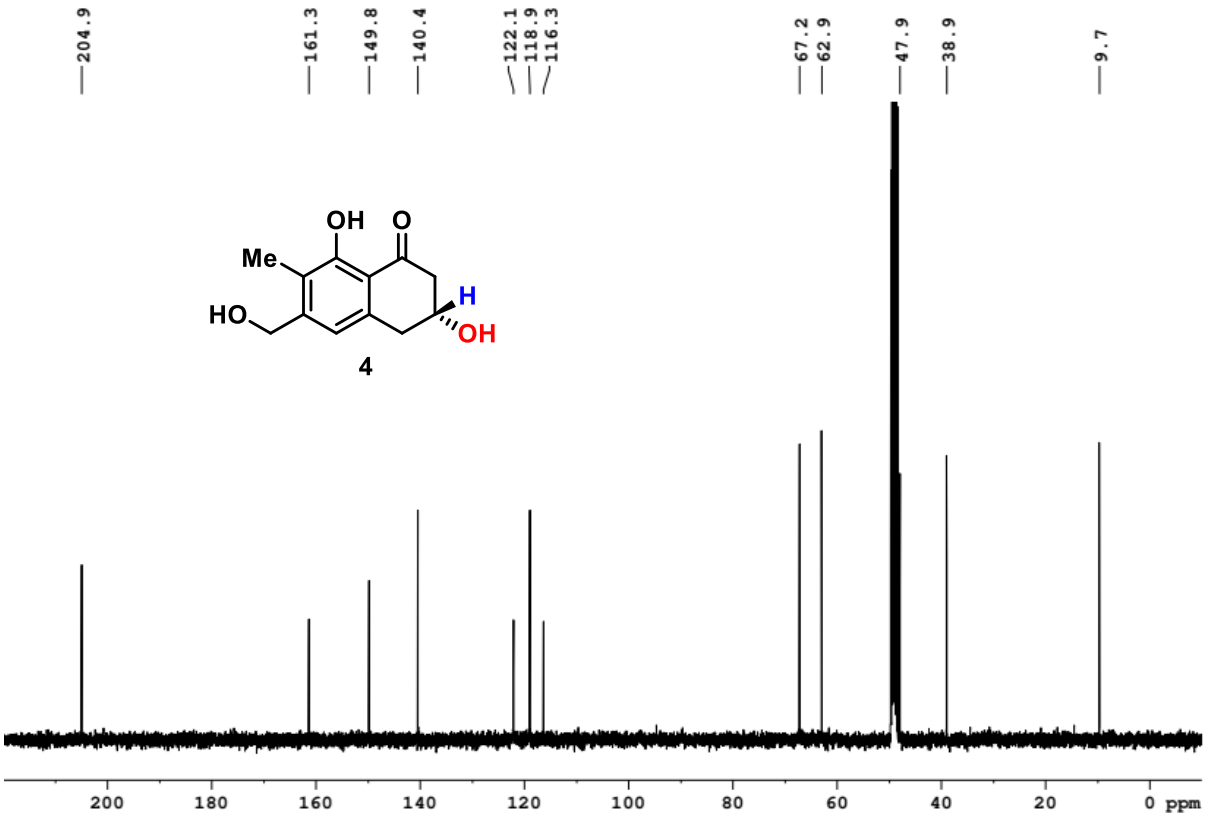
^{1}H-^{13}C HSQC (400 MHz, acetone-d_6)



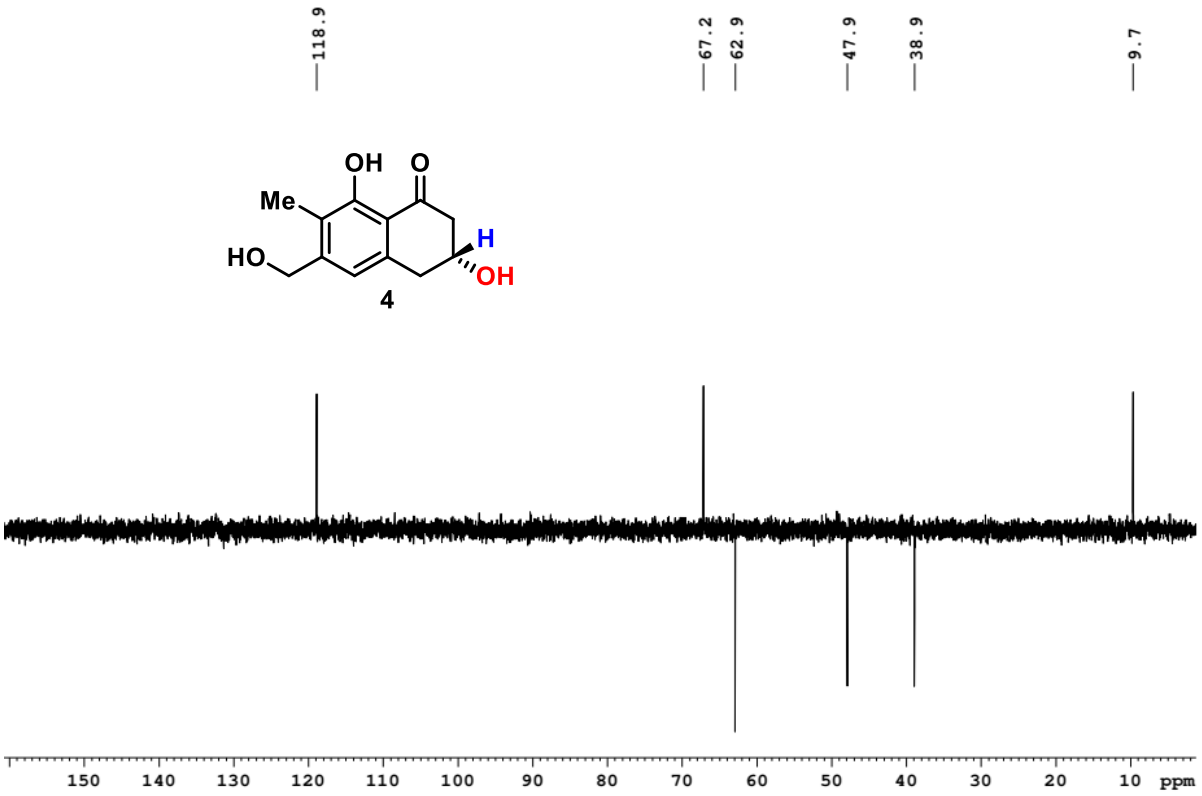
^{1}H NMR (400 MHz, methanol-d_4)



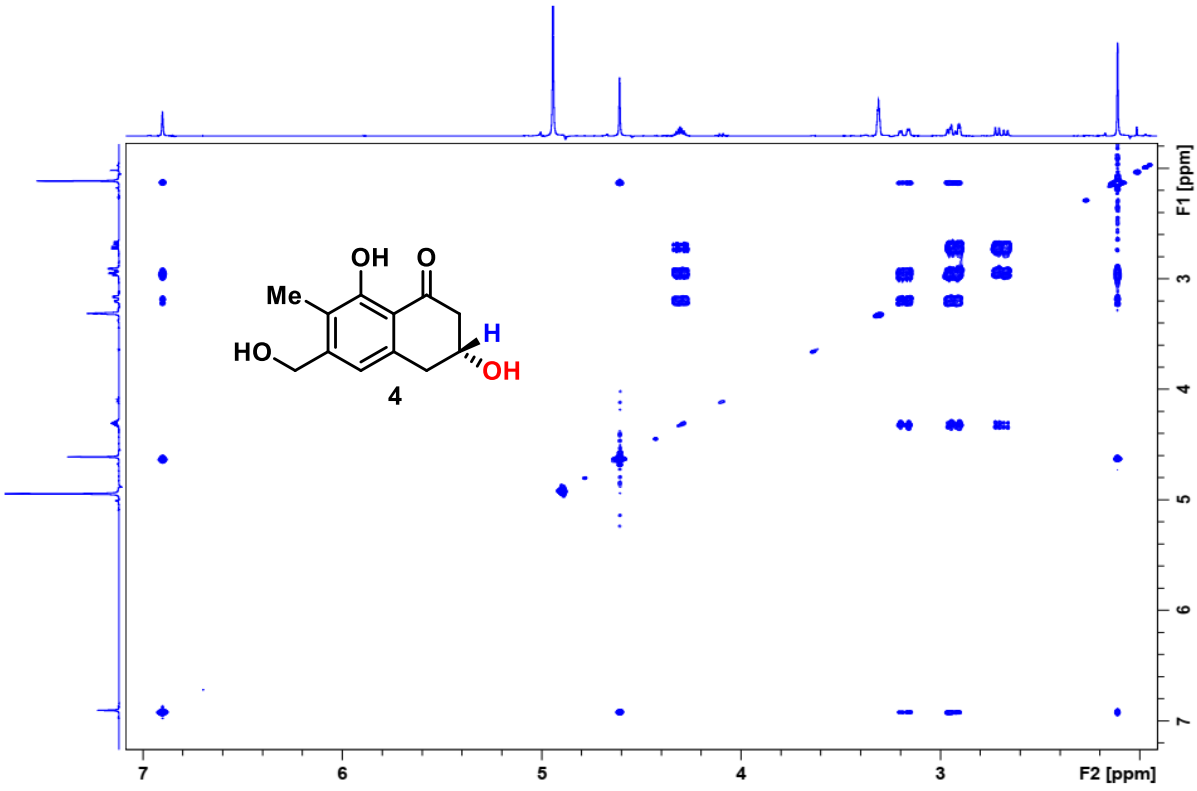
^{13}C NMR (100 MHz, methanol-d_4)



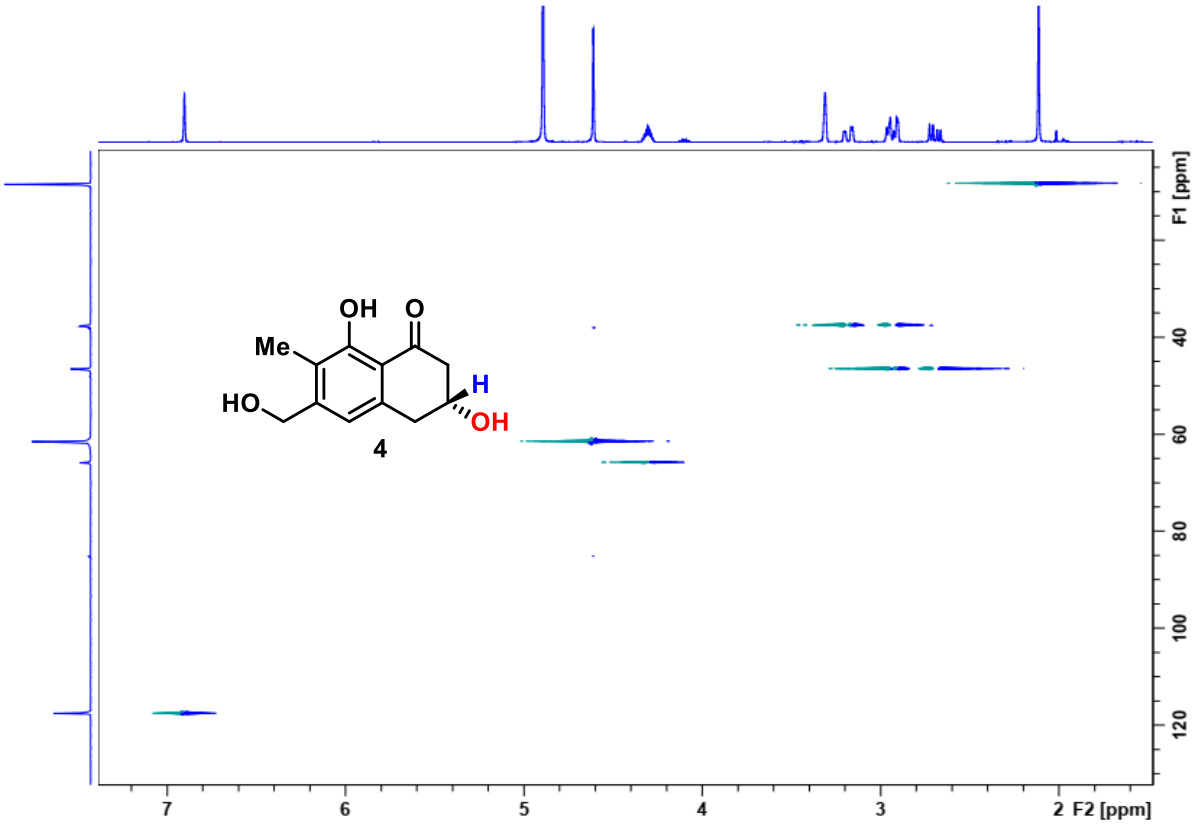
DEPT-135 NMR (100 MHz, methanol-d_4)



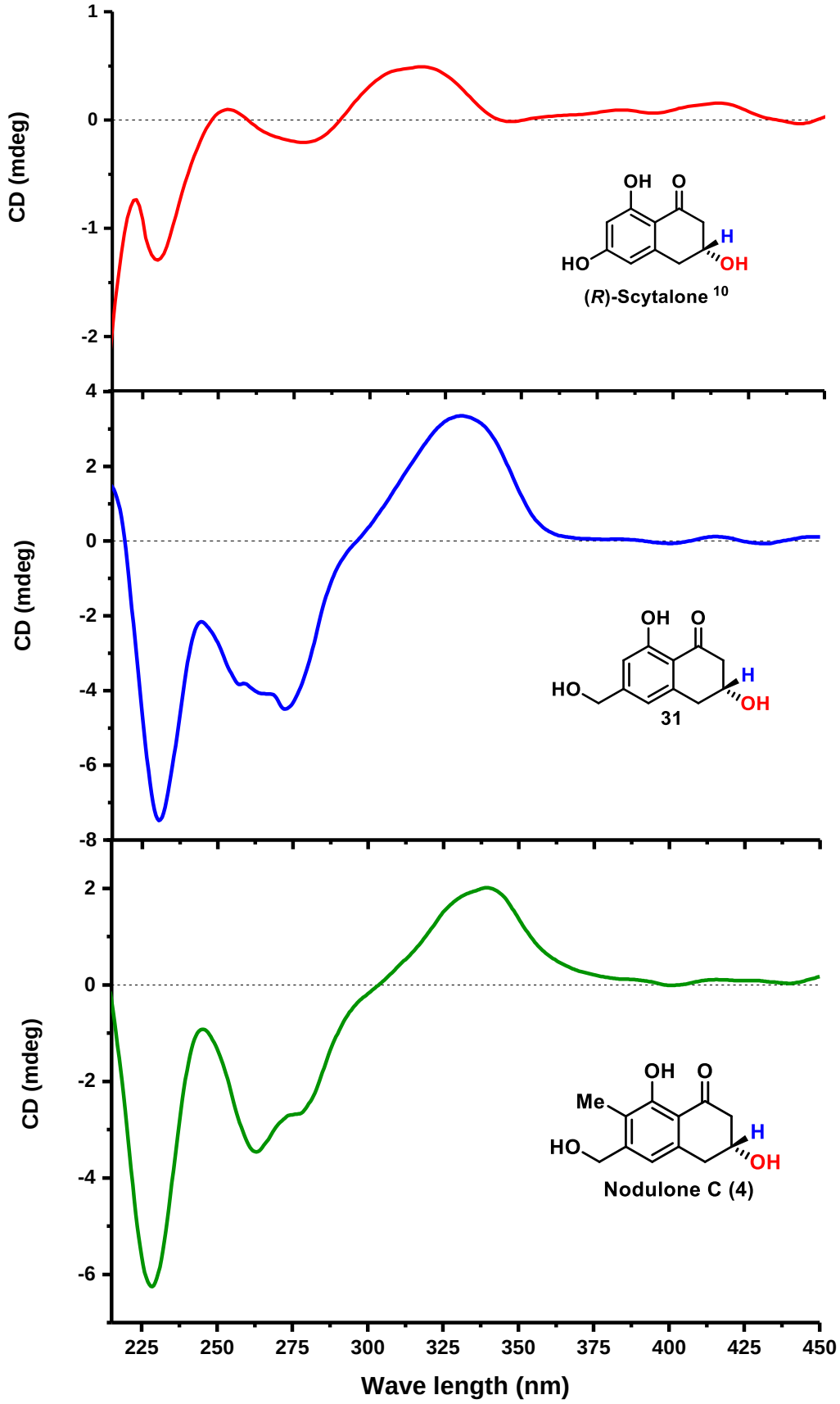
^{1}H-^{1}H COSY (400 MHz, methanol-d_4)

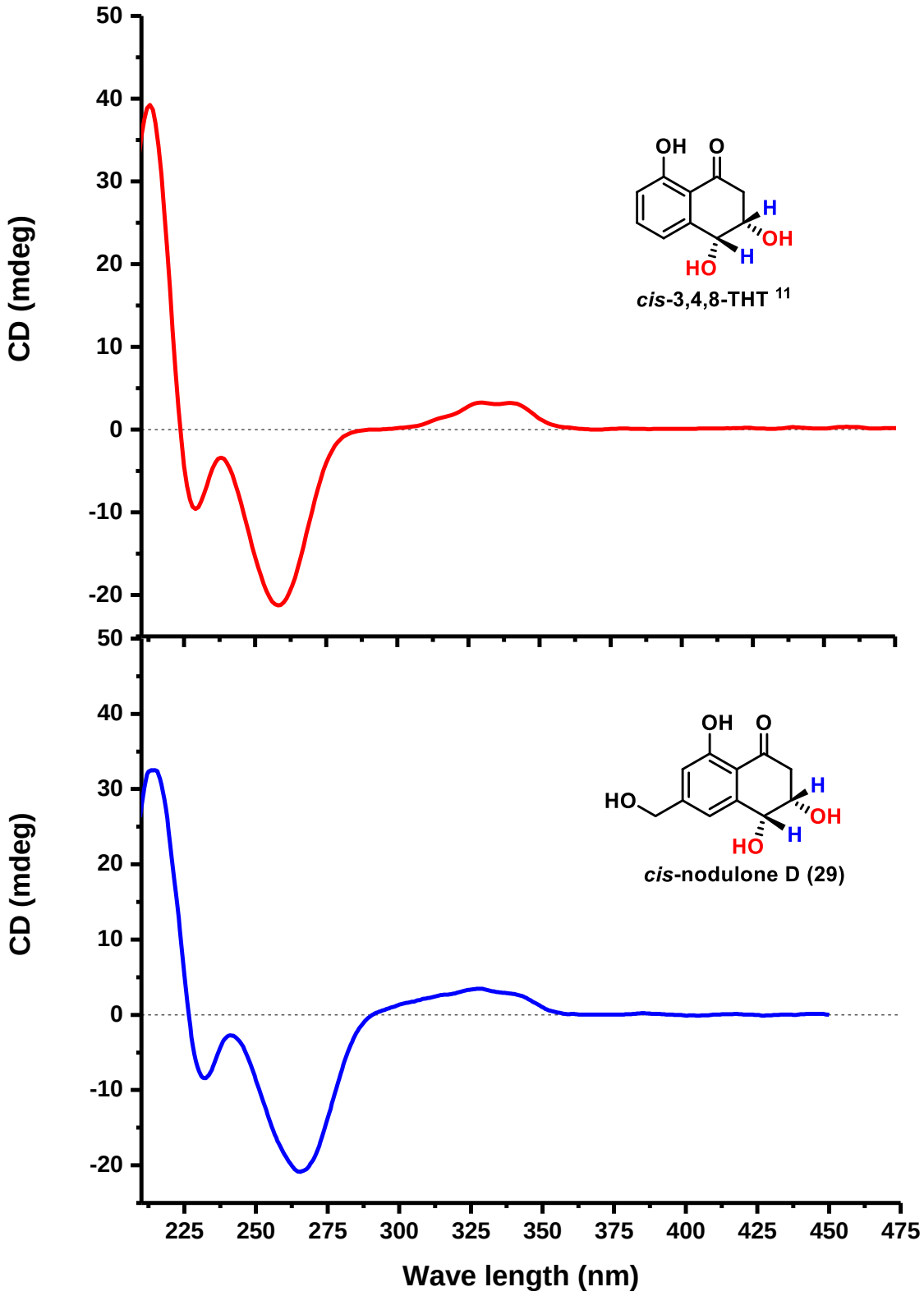


^{1}H-^{13}C HSQC (400 MHz, methanol-*d*$_4$)



VIII. Circular Dichroism (CD) Spectra




CD (mdeg)
50
40
30
20
10
0
-10
-20
OH
O
H
OH
HO
H
cis-3,4,8-THT 11
CD (mdeg)
50
40
30
20
10
0
-10
-20
OH
O
HO
H
OH
HO
H
cis-nodulone D (29)
225
250
275
300
325
350
375
400
425
450
475
Wave length (nm)

IX. References

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