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Supporting information

Chemo-Enzymatic Three-Step Conversion of Glucose to Kojic Acid

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Experimental Procedures

General

For identification and characterization of compounds a Bruker Avance-III spectrometer operating at 500.20 MHz (^{1}H) and 125.78 MHz (^{13}C) equipped with a Prodigy BBO (CryoProbe) was used. The characterization was performed using the following 1D NMR spectroscopic techniques: ^{1}H and ^{13}C NMR combined with 2D DQF-COSY, HSQC, and HMBC. TLC analysis was performed on Merck silica gel 60 F_{254} plates and the spots were visualized with UV light and charring with H_2SO_4/MeOH (1:4) and heating. Column chromatography was carried out using silica gel 60 (0.040 – 0.060 mm) as stationary phase and hexane/EtOAc as the eluents. HRMS recorded with a Bruker Daltonics micrOTOF-Q with ESI in positive mode as ionization source. GC-EIMS recorded on an Agilent Technologies 7890A GC-system equipped with an Agilent J&W HP-5ms GC Column (30 m x 0.25 mm, 0.25 µm film) and an Agilent Technologies 5975C EIMS-detector. Silylation of kojic acid was done using hexamethyldisilazane (HMDS) and chlorotrimethylsilane (TMCS) in pyridine prior to analysis.

Chromatographic analysis of glucose to glucosone conversion was performed using Water HPLC instrument with Breeze 2 software equipped with refraction index detector and Waters High Performance Carbohydrate Column (Waters P/N WAT044355; 4.6 x 250 mm column packed with 4-µm spherical silica). Chromatography was carried out in isocratic mode with 75% acetonitrile in water, with 1.4 ml/min solvent flow rate, at 35 °C. Samples were prepared in 60% acetonitrile and filtered with 0.2 micron filter. Sample volume was 8 µl, run time 8 min

*Bioconversion of glucose (**2**) to glucosone (**3**):*

Enzymatic conversion of glucose to glucosone was carried out in 1 l twin-pot bioreactor (BIOSTAT® B plus twin from Sartorius Stedim Biotech). The bioconversion was performed in 500 ml reaction mixture containing 45 g D-glucose (**2**) (Sigma-Aldrich Cat#1.08337), resulting in a final concentration of 500 mM; 50 mL MetZyme® PPURECO Pyranose Oxidase (10 U/ml, MetGen, Finland) and 2 ml of catalase (130 u/ml, Habio, China). The reaction was carried out at 45 °C with constant aeration (dissolved oxygen level maintained at 20 – 30%) and pH maintained at 6.0 with ammonium hydroxide. The reaction was continued for 10 h. Next, the reaction mixture was centrifuged 10 000 g for 30 min and the pellet, containing pyranose oxidase enzyme, was discarded and the supernatant, containing glucosone, was filtered through 0.45 microne filter and lyophilized. Yield: >95%.

*Di-O-acetyl-kojic acid (**4**) and (2R,3R)-3,5-diacetoxy-2-(acetoxymethyl)-2,3-dihydro-4H-pyran-4-one (**5**):*

Glucosone (710 mg, 1 eq) was dissolved in DMF (18 ml). NaOAc (1.6 g, 5 eq) and Ac_2O (2.3 ml, 6 eq) were added under stirring. After 22 h, the reaction was quenched by the addition of MeOH. After 15 min, the reaction mixture was diluted with CH_2Cl_2 (120 ml) and washed with saturated $NaHCO_3$ solution (100 ml) and saturated NaCl solution (80 ml). Next, the organic phase was dried over Na_2SO_4 and the solvent evaporated. The product was purified by column chromatography (hexane:EtOAc 1:2).

4: Yield: 407 mg (45%). R_f = 0.32 (hexane/EtOAc 1:2), ^{1}H NMR (500.20 MHz, $CDCl_3$, 25 °C): δ = 7.89 (d, 1 H, H-1), 6.50 (dd, 1 H, $J_{H\text{-}4,H\text{-}6}$ = 0.6 Hz, H-4), 4.92 (dd, 2 H, H-6), 2.33 (s, 3 H, 2-OCOCH_3), 2.16 (s, 3 H, 6-OCOCH_3) ppm. ^{13}C NMR (125.8 MHz, $CDCl_3$,

25 °C): δ = 172.3 (C-3), 169.8 (6-O*C*OCH$_3$), 167.7 (2-O*C*OCH$_3$), 162.4 (C-5), 147.9 (C-1), 141.3 (C-2), 115.2 (C-4), 60.9 (C-6), 20.5 (6-OCO*C*H$_3$), 20.4 (2-OCO*C*H$_3$) ppm. HRMS: calculated for $C_{10}H_{11}O_6Na$, $[M + Na]^+$ 249.0375 and measured 249.0362.

5: Yield: 340 mg (30%). R_f = 0.50 (hexane/EtOAc 1:2), ^{1}H NMR (500.20 MHz, CDCl$_3$, 25 °C): δ = 7.47 (d, 1 H, $J_{H\text{-}1,H\text{-}5}$ = 0.6 Hz, H-1), 5.70 (d, 1 H, $J_{H\text{-}4,H\text{-}5}$ = 13.0 Hz, H-4), 4.72 (dddd, 1 H, $J_{H\text{-}5,H\text{-}6a}$ = 4.1 Hz, $J_{H\text{-}5,H\text{-}6b}$ = 2.3 Hz, H-5), 4.45 (dd, 1 H, $J_{H\text{-}6a,H\text{-}6b}$ = –12.9 Hz, H-6a), 4.45 (dd, 1 H, H-6b), 2.26 (s, 3 H, 1-OCOC*H$_3$*), 2.21 (s, 3 H, 4-OCOC*H$_3$*), 2.15 (s, 3 H, 6-OCOC*H$_3$*) ppm. ^{13}C NMR (125.8 MHz, CDCl$_3$, 25 °C): δ = 181.9 (C-3), 170.3 (6-O*C*OCH$_3$), 168.9 (4-O*C*OCH$_3$), 168.5 (2-O*C*OCH$_3$), 155.3 (C-1), 130.5 (C-2), 79.0 (C-5), 67.7 (C-4), 61.2 (C-6), 20.6 (6-OCO*C*H$_3$), 20.4 (4-OCO*C*H$_3$), 20.1 (2-OCO*C*H$_3$) ppm. HRMS: calculated for $C_{12}H_{14}O_8Na$, $[M + Na]^+$ 309.0581 and measured 309.0588.

*Kojic acid (**1**):*

To a solution of **4** (310 mg, 1 eq) in MeOH (5 ml) was added 5.4 M NaOMe in MeOH (250 µl, 1 eq). After 1 h, the reaction was stopped by addition of DOWEX 50 until the pH was neutral. Next, the mixture was filtered and the solvent was evaporated. Yield: 95%. R_f = 0.30 (CH_2Cl_2/MeOH 10:1). ^{1}H NMR (500.20 MHz, MeOD, 25 °C): δ = 7.97 (d, 1 H, H-1), 6.52 (dd, 1 H, $J_{H\text{-}4,H\text{-}6}$ = 0.6 Hz, H-4), 4.43 (dd, 2 H, H-6) ppm. ^{13}C NMR (125.8 MHz, MeOD, 25 °C): δ = 176.9.4 (C-3), 170.4 (C-5), 147.4 (C-2), 141.0 (C-4), 110.7 (C-1), 61.2 (C-6) ppm.

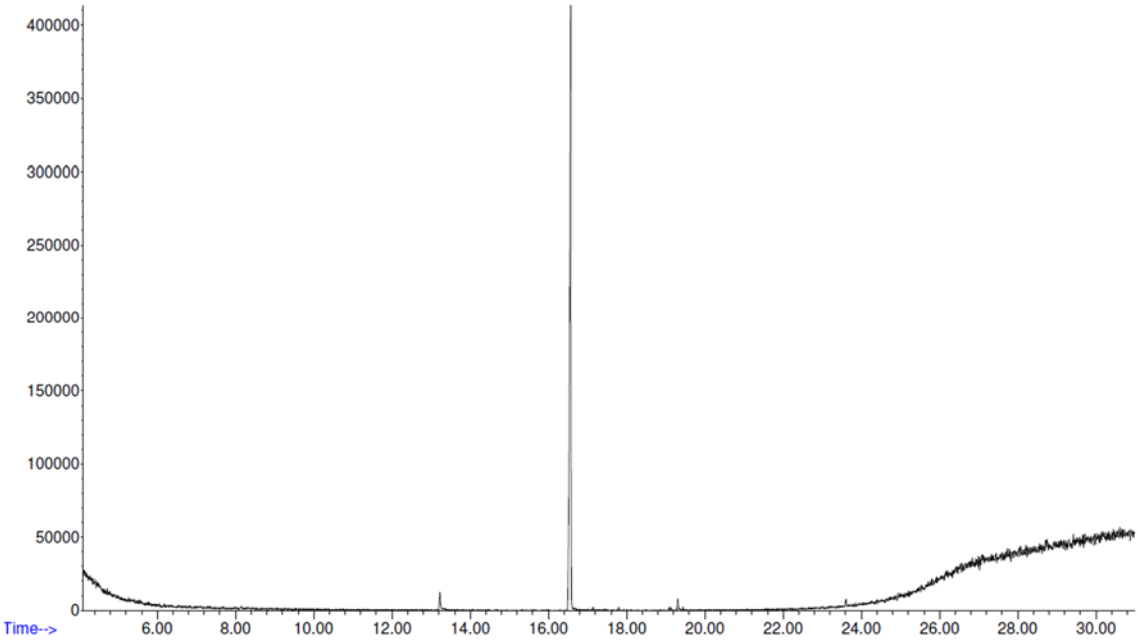
*Direct synthesis of kojic acid (**1**) from glucosone (**3**):*

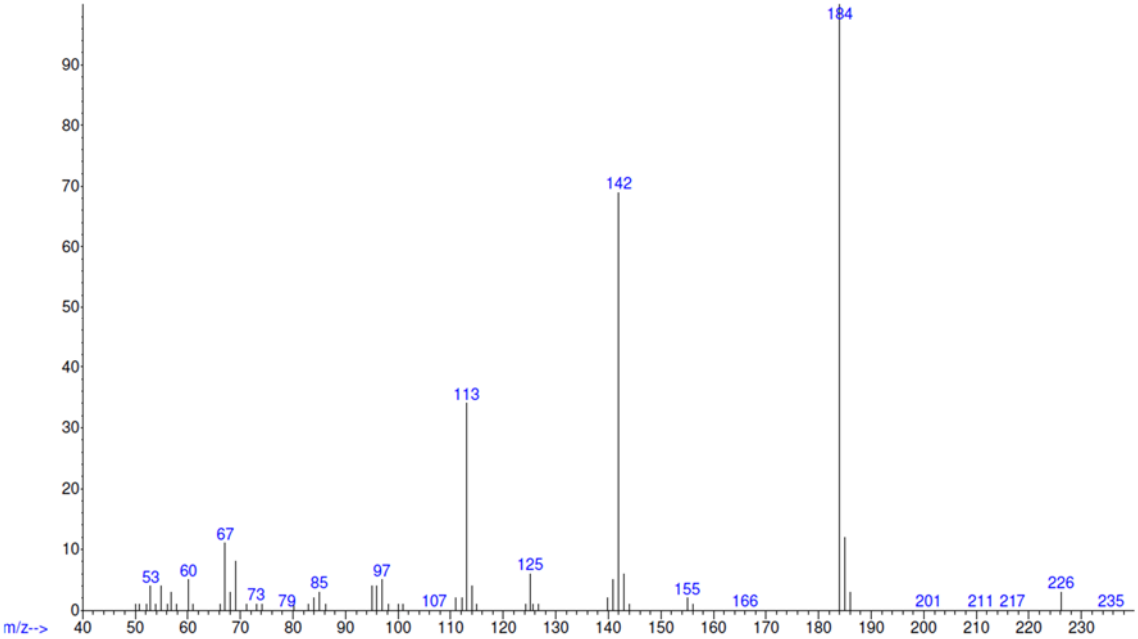
To a solution of glucosone (**3**) (1.3 g, 1 eq) in DMF (32 ml) were added NaOAc (3.0 g, 5 eq) and Ac_2O (4.2 ml, 6 eq). After 23 h, the reaction was quenched by the addition of MeOH. After 15 min, the reaction mixture was diluted with CH_2Cl_2 (250 ml) and washed with saturated $NaHCO_3$ solution (200 ml) and saturated NaCl solution (150 ml). The organic phase was dried over Na_2SO_4 and the solvent evaporated. The product was dissolved in MeOH (30 ml) and 5.4 M NaOMe in MeOH (1.4 ml, 1 eq) was added. After 1 h, the reaction was stopped by addition of DOWEX 50 until the pH was neutral. The mixture was filtered and the solvent evaporated. Kojic acid was extracted from the crude mixture with EtOH. Impurities were precipitated from this mixture by the addition of acetone. The mixture was filtered and the solvent evaporated. The remaining impurities were dissolved in acetone and the mixture was again filtered giving **1** as a slightly yellow powder. Yield: 200 mg (20%).

GC-MS

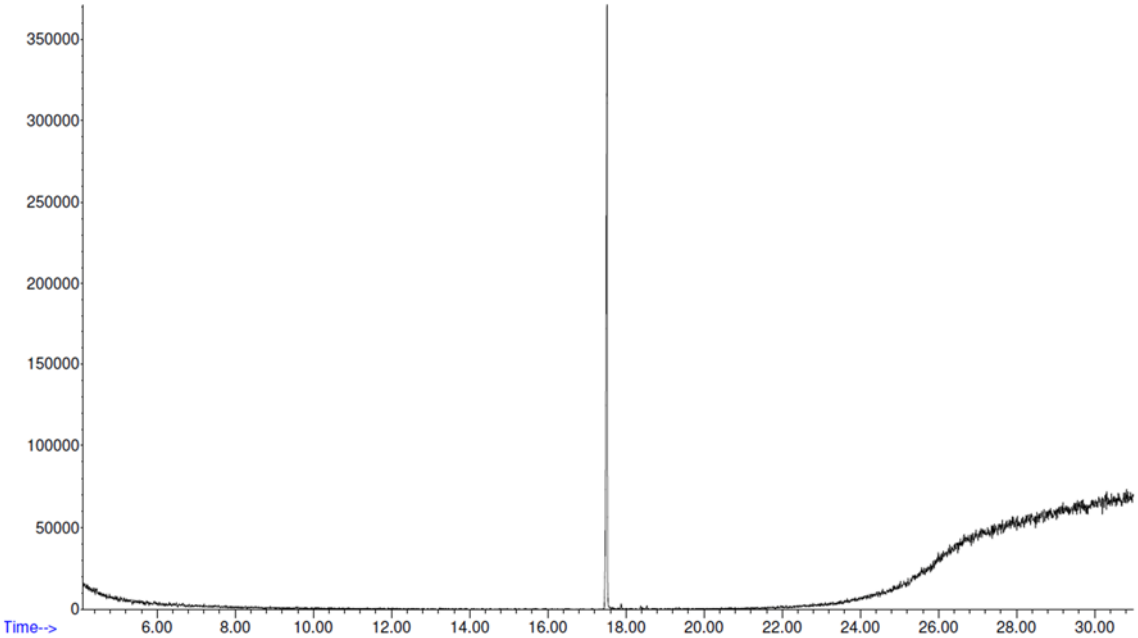
Di-*O*-acetyl kojic acid (4)

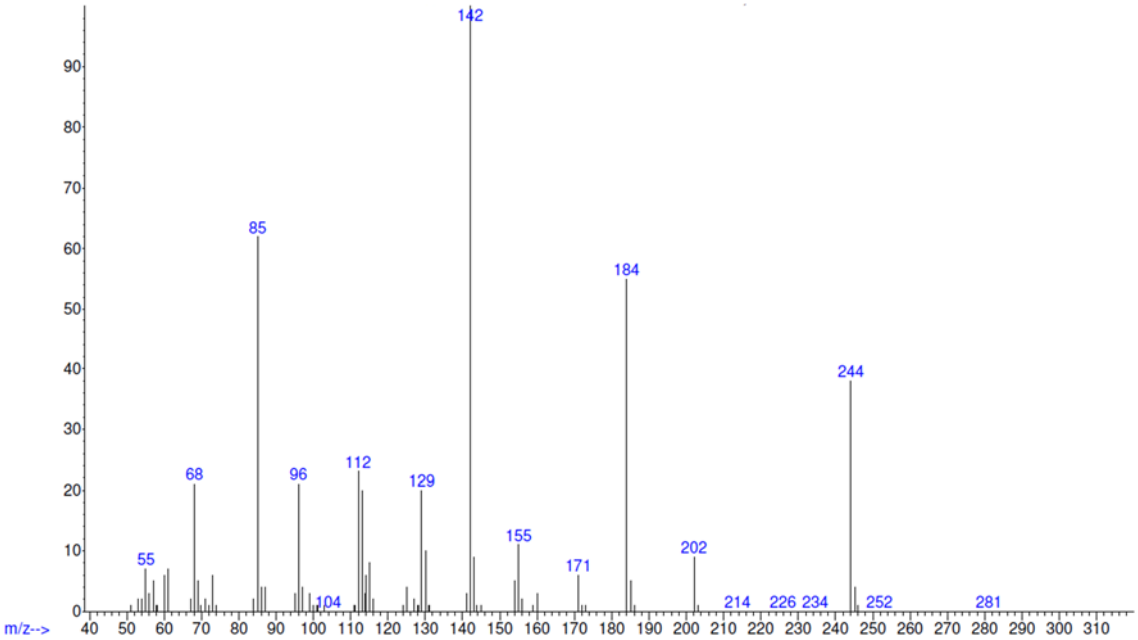
[M^+= 226]



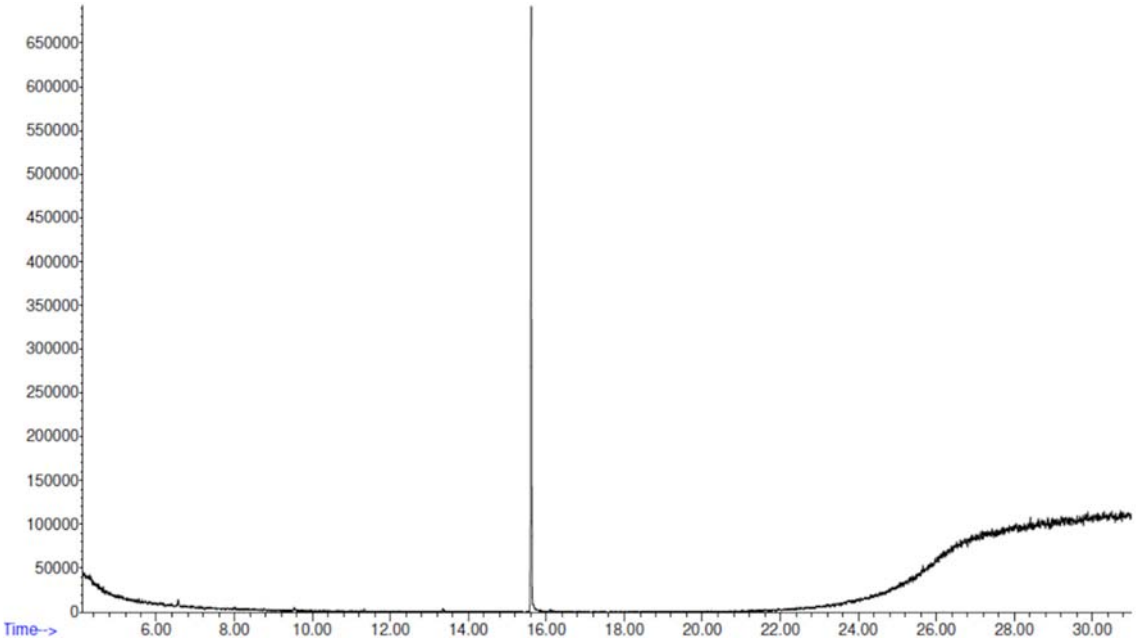


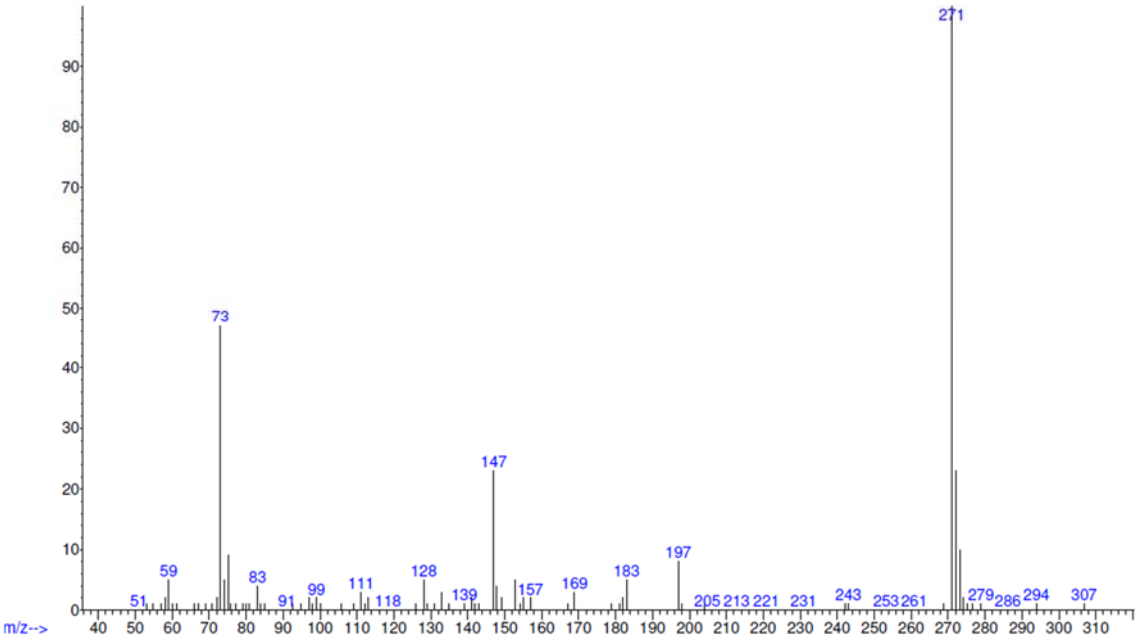
(2*R*,3*R*)-3,5-Diacetoxy-2-(acetoxymethyl)-2,3-dihydro-4H-pyran-4-one (5)
[M^+= 286]





Kojic acid (1) (Silylated) (from the crude **4**/**5** mixture)
[M^{+}= 286]

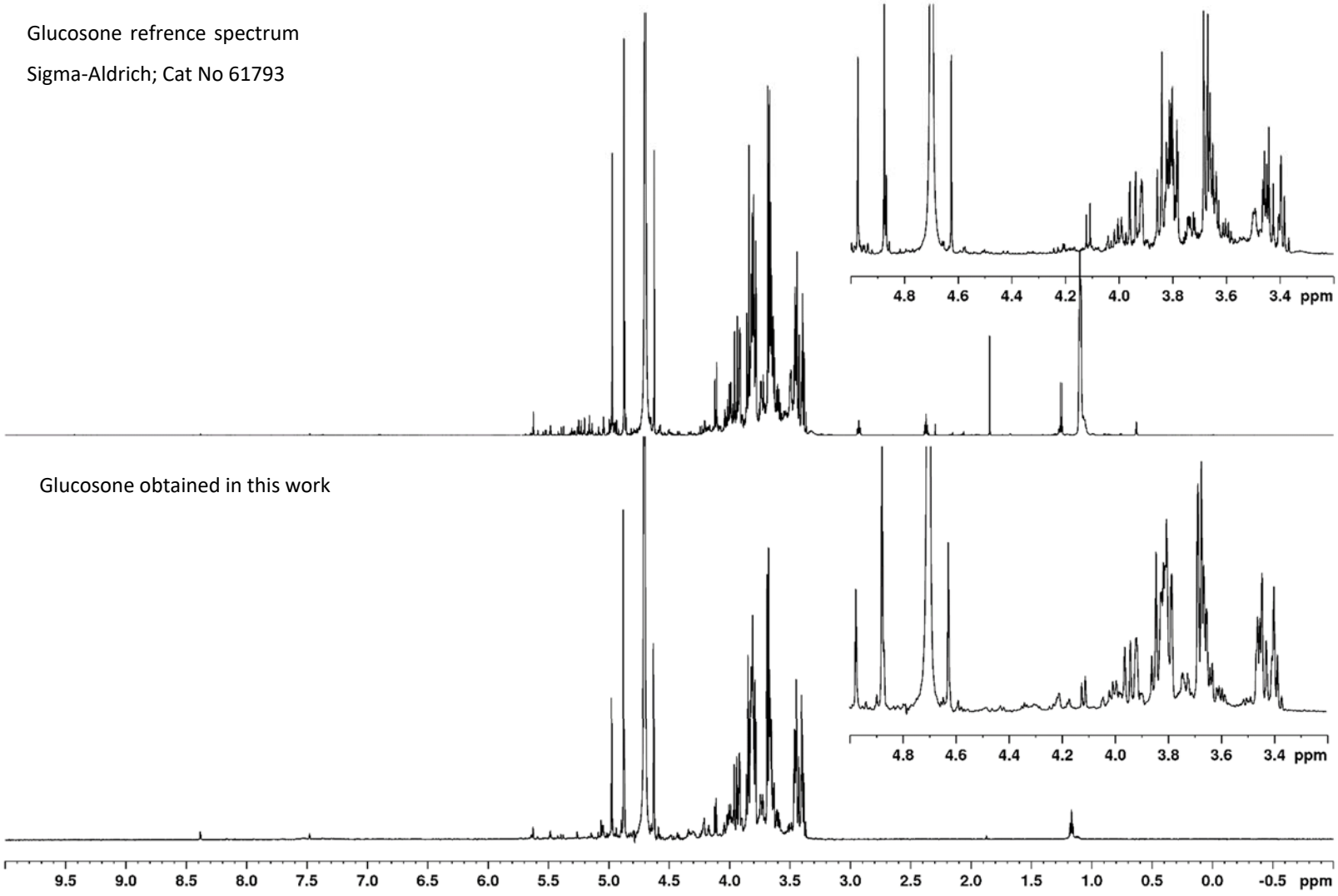




NMR Spectra

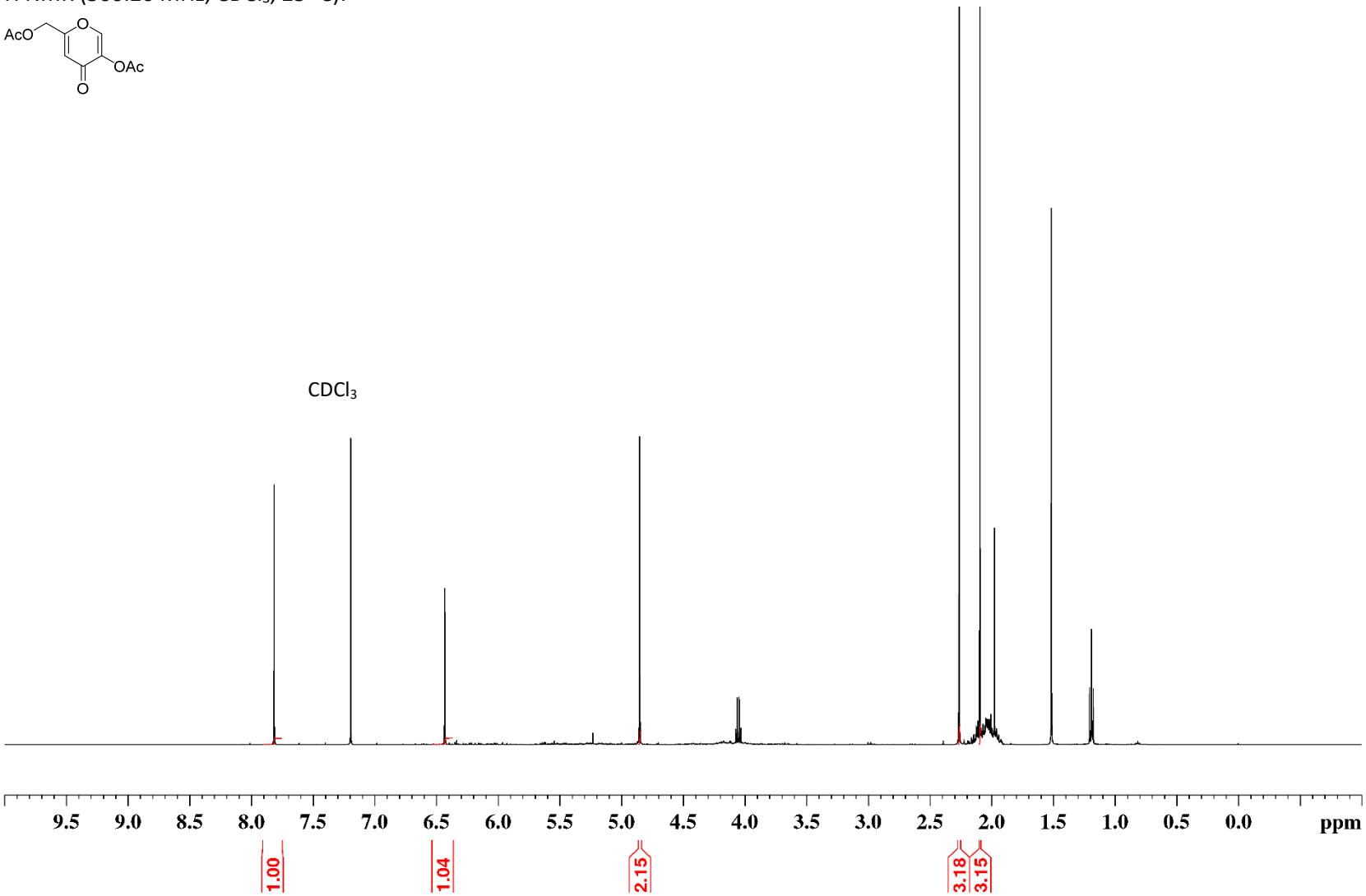
Glucosone (3)

^{1}H NMR (500.20 MHz, D_2O, 25 °C):

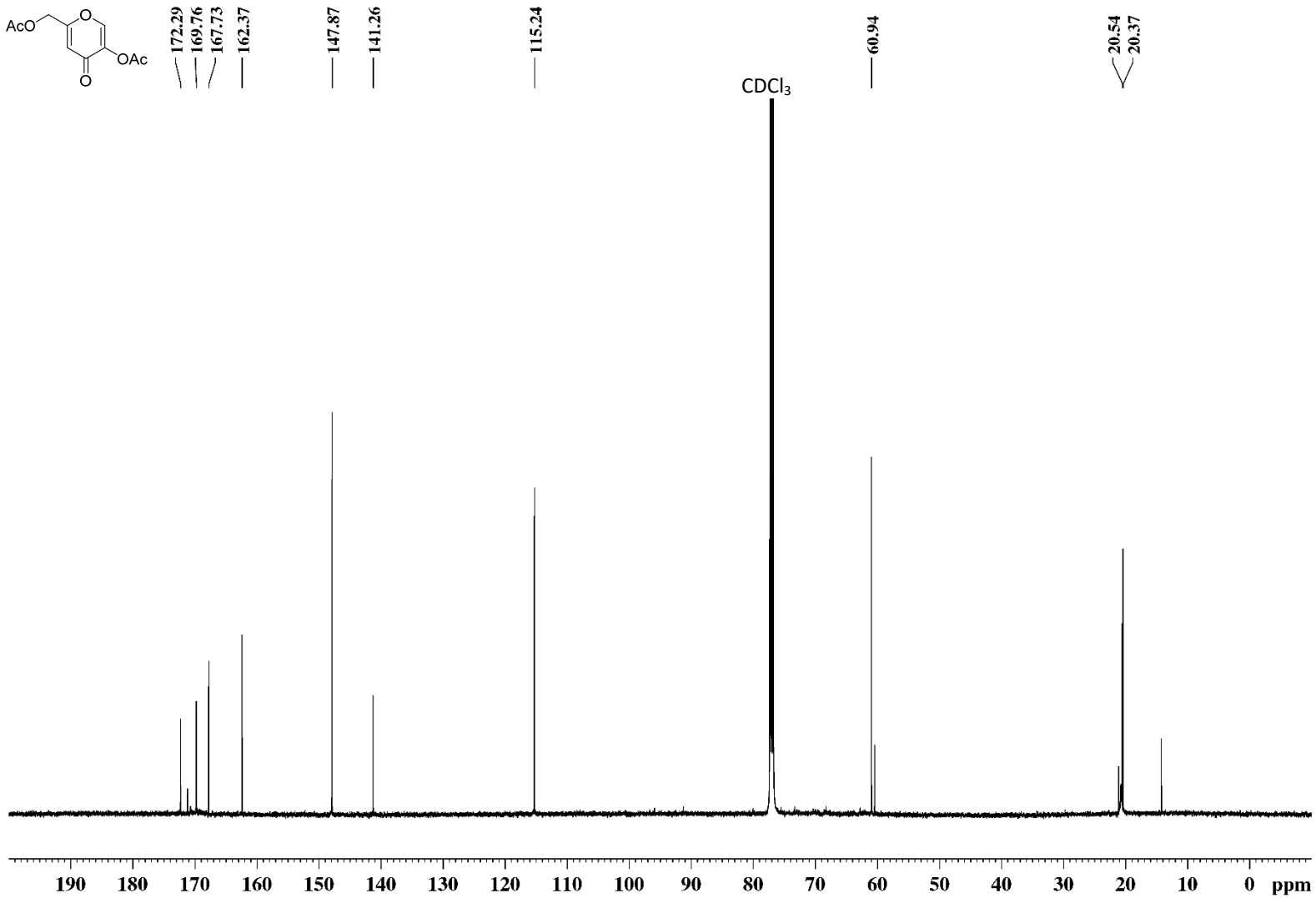


Di-*O*-acetyl kojic acid (4)

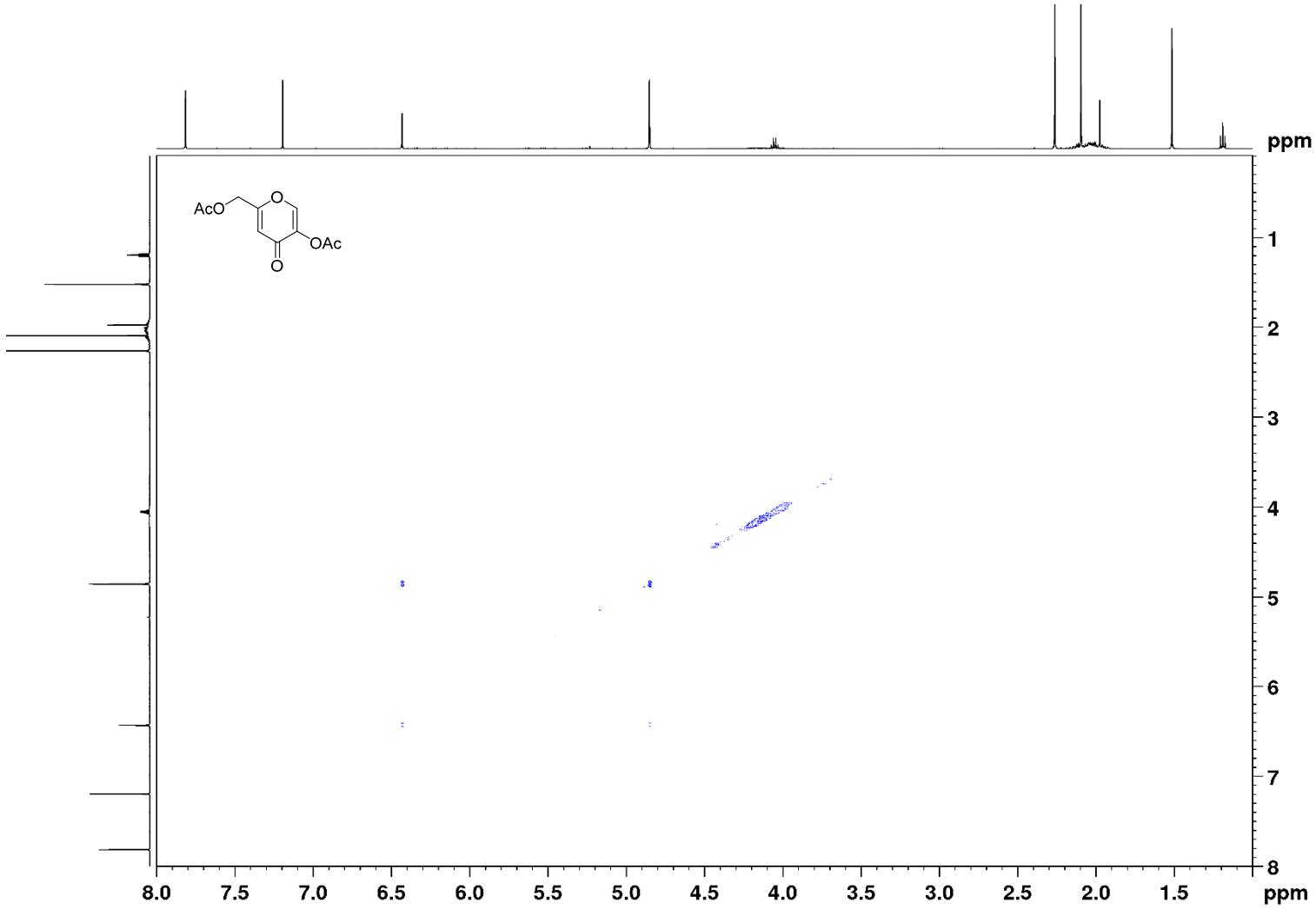
^{1}H NMR (500.20 MHz, $CDCl_3$, 25 °C):



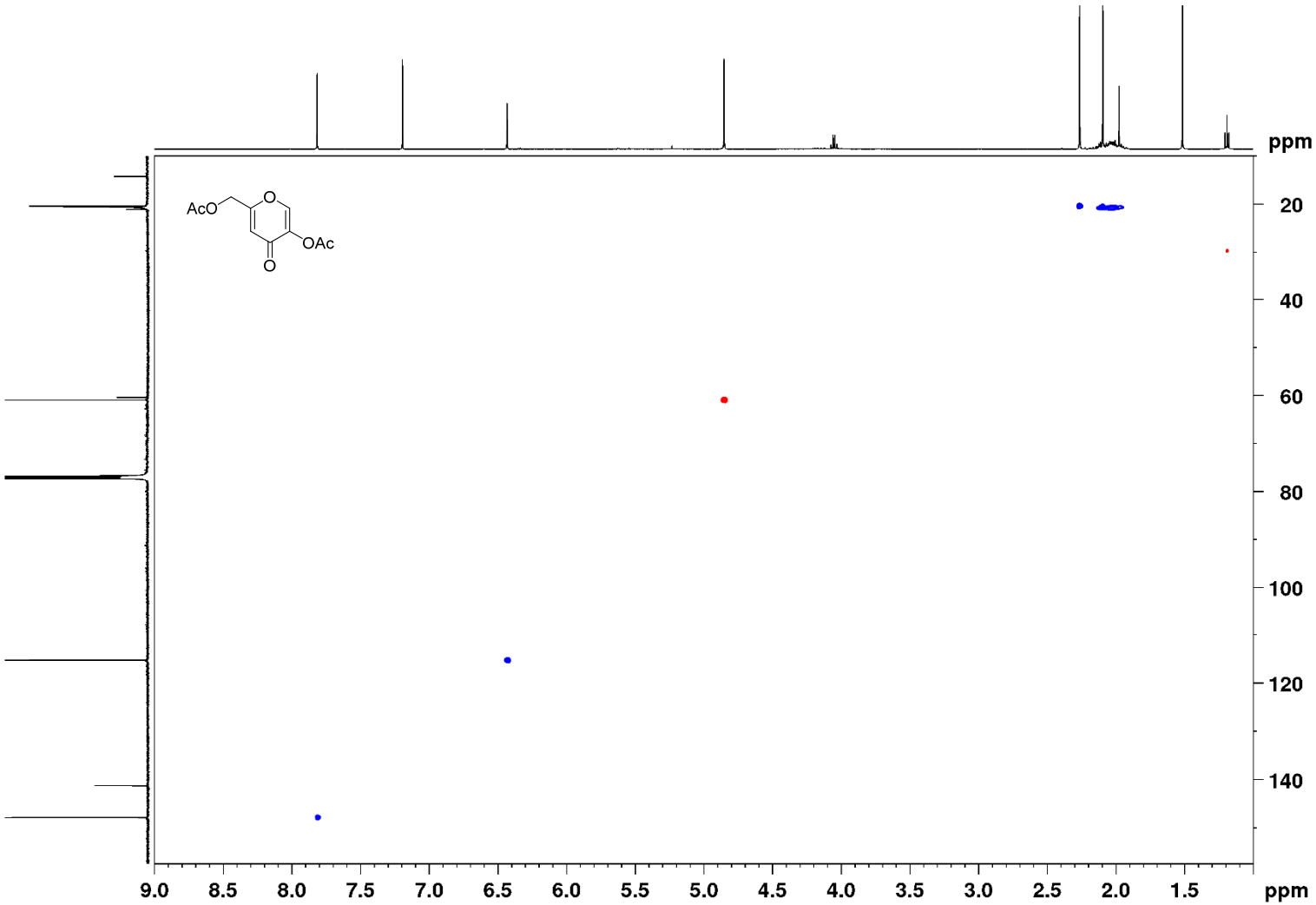
^{13}C NMR (125.8 MHz, $CDCl_3$, 25 °C):



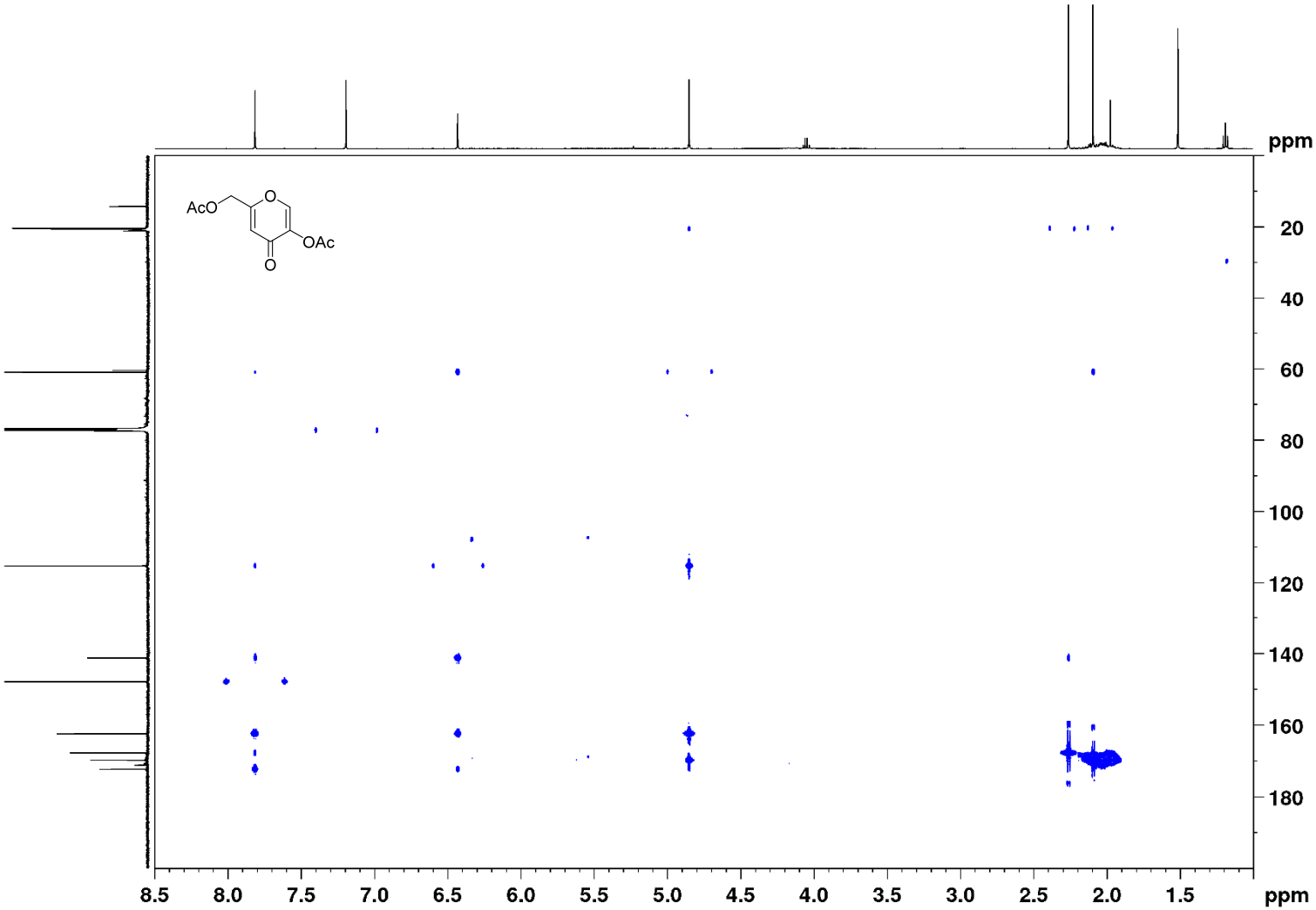
DQF-COSY NMR ($CDCl_3$, 25 °C):



HSQC NMR ($CDCl_3$, 25 °C):

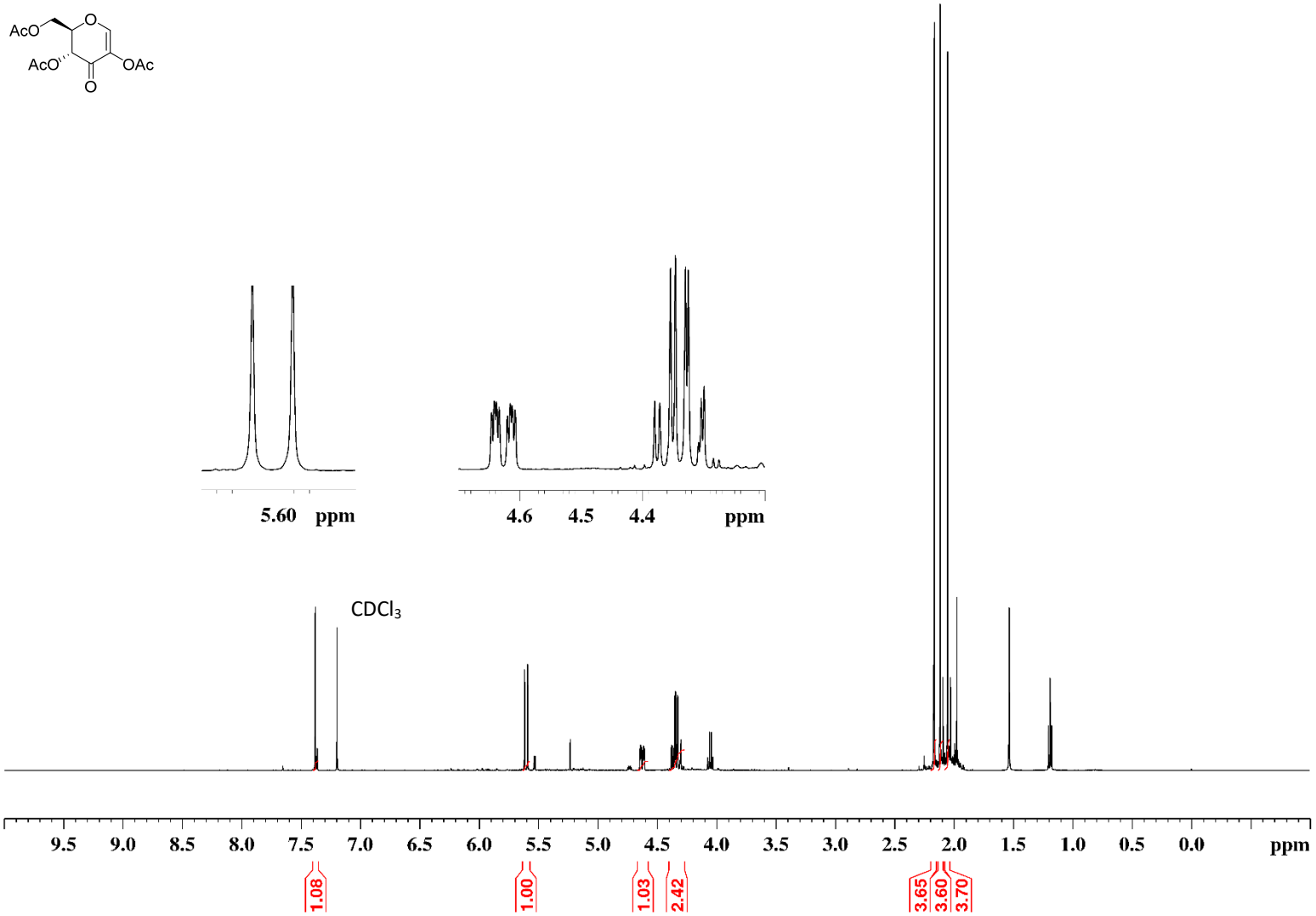


HMBC NMR ($CDCl_3$, 25 °C):

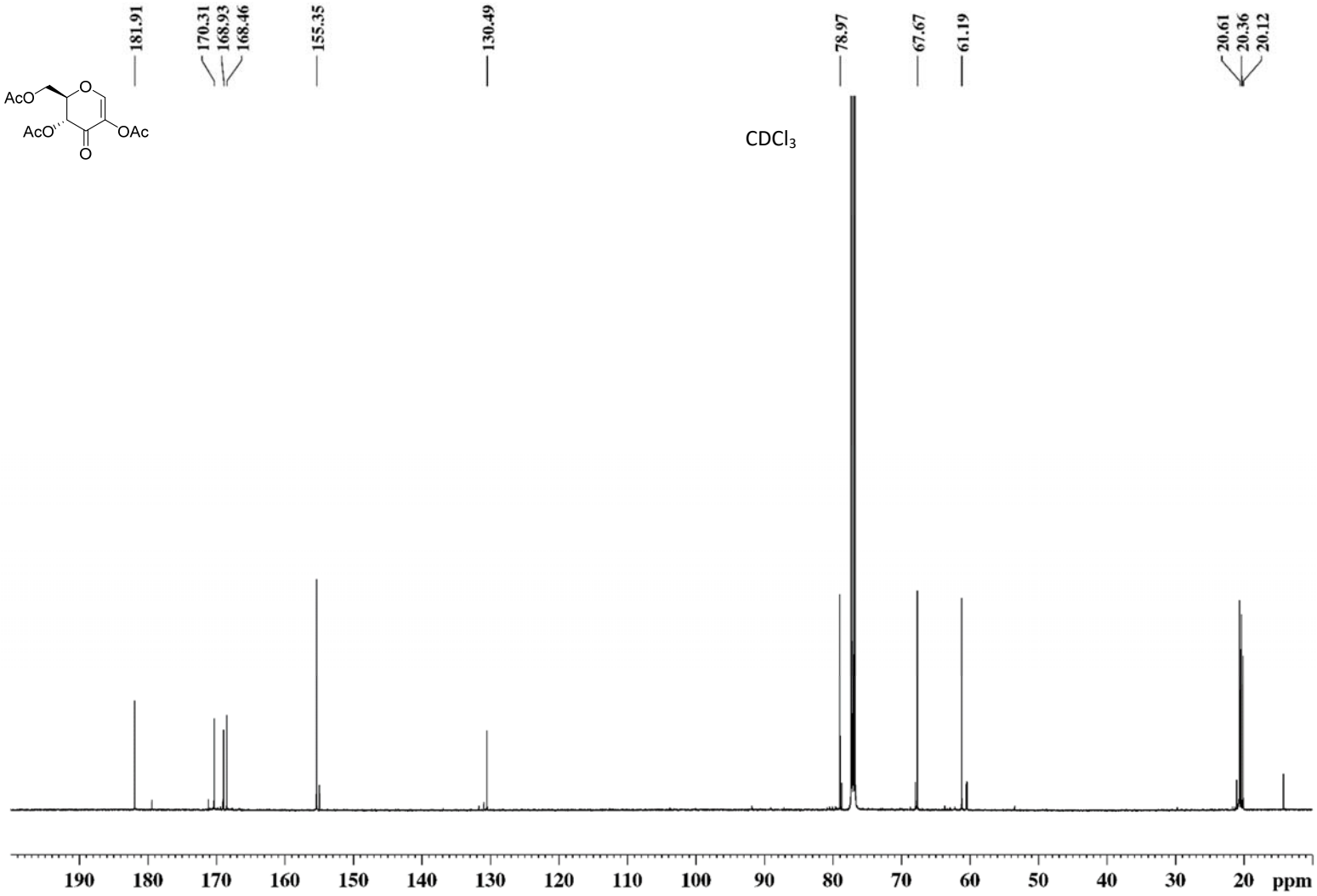


(2*R*,3*R*)-3,5-Diacetoxy-2-(acetoxymethyl)-2,3-dihydro-4H-pyran-4-one (5)

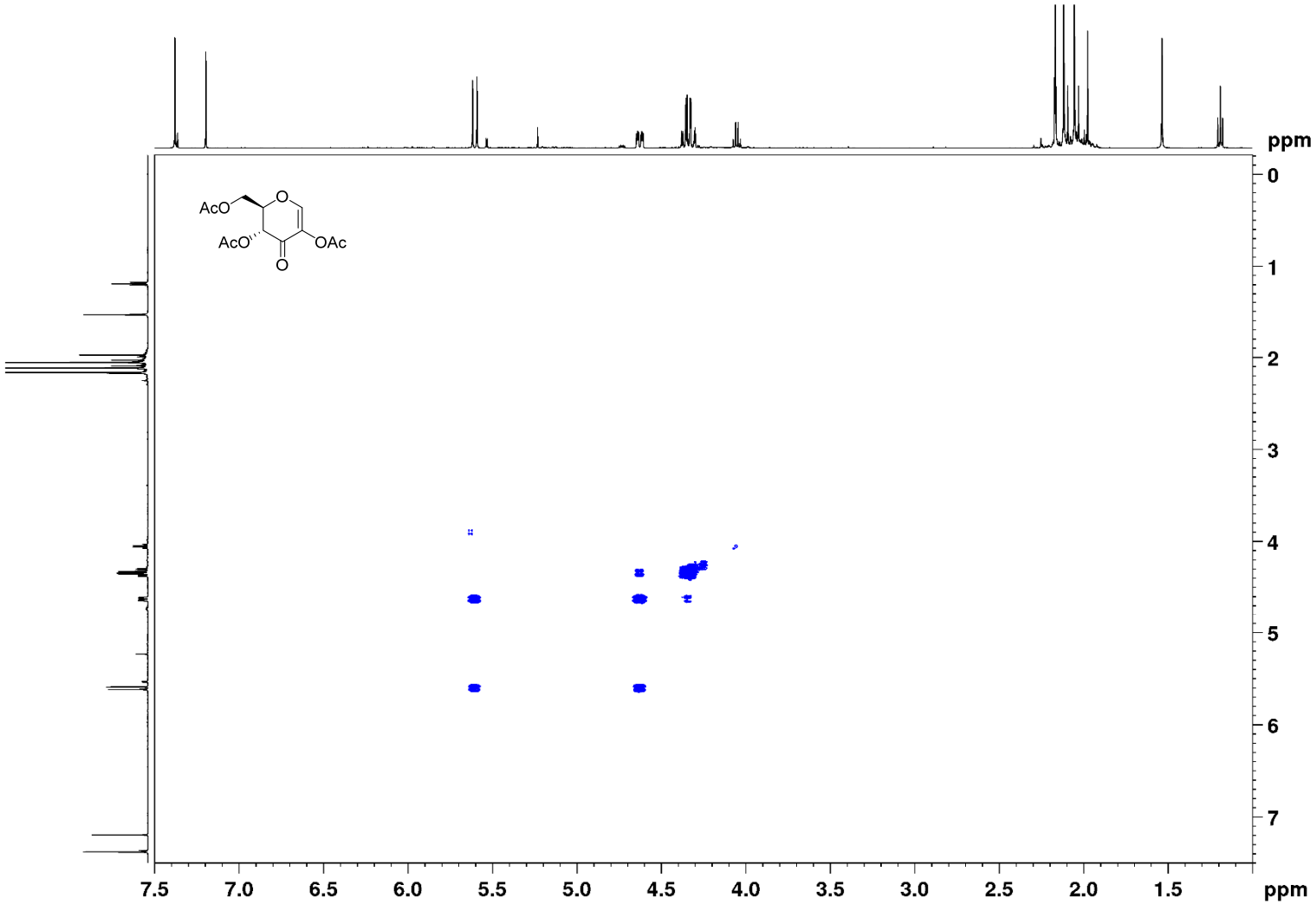
^{1}H NMR (500.20 MHz, $CDCl_3$, 25 °C):



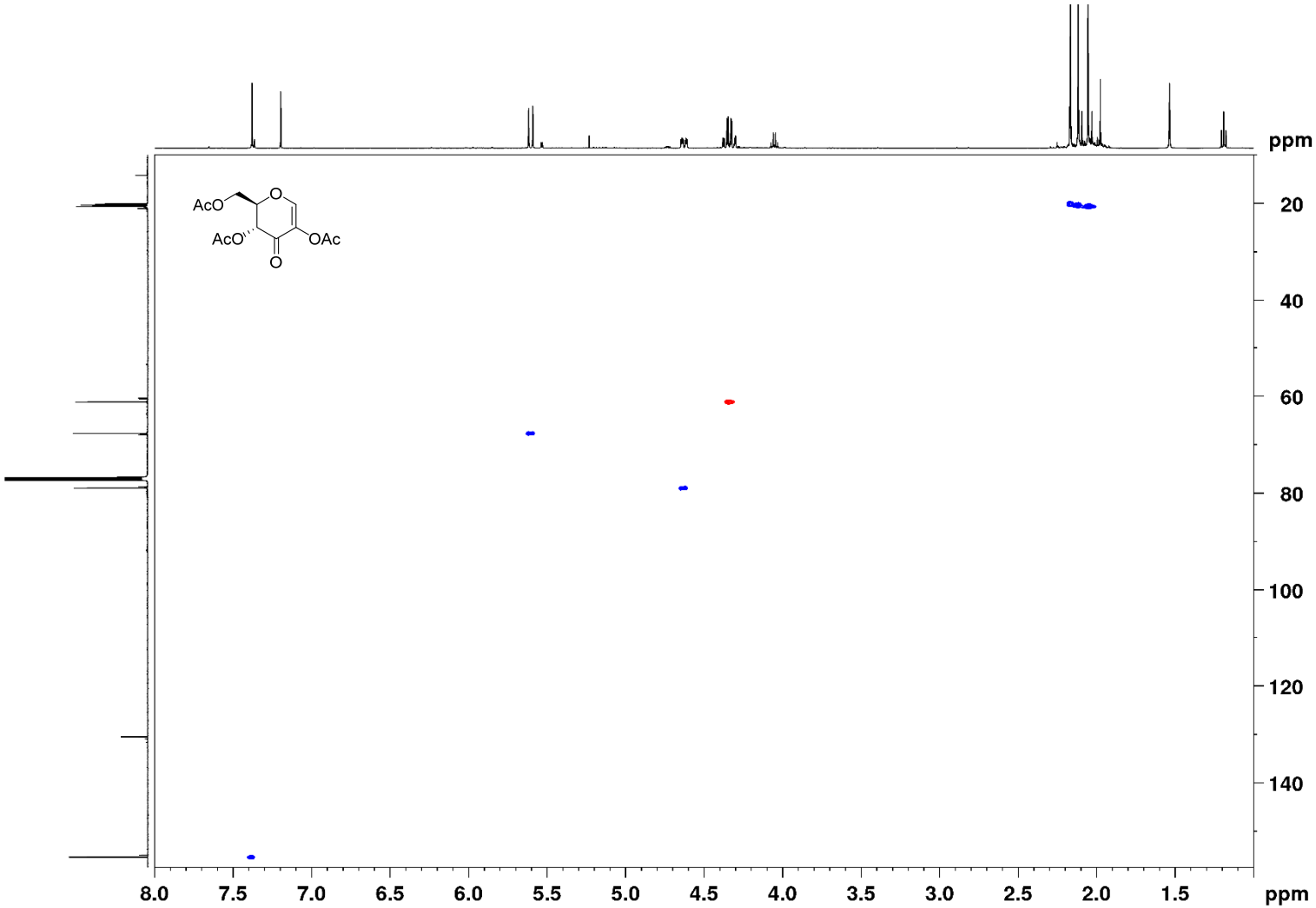
^{13}C NMR (125.8 MHz, $CDCl_3$, 25 °C):



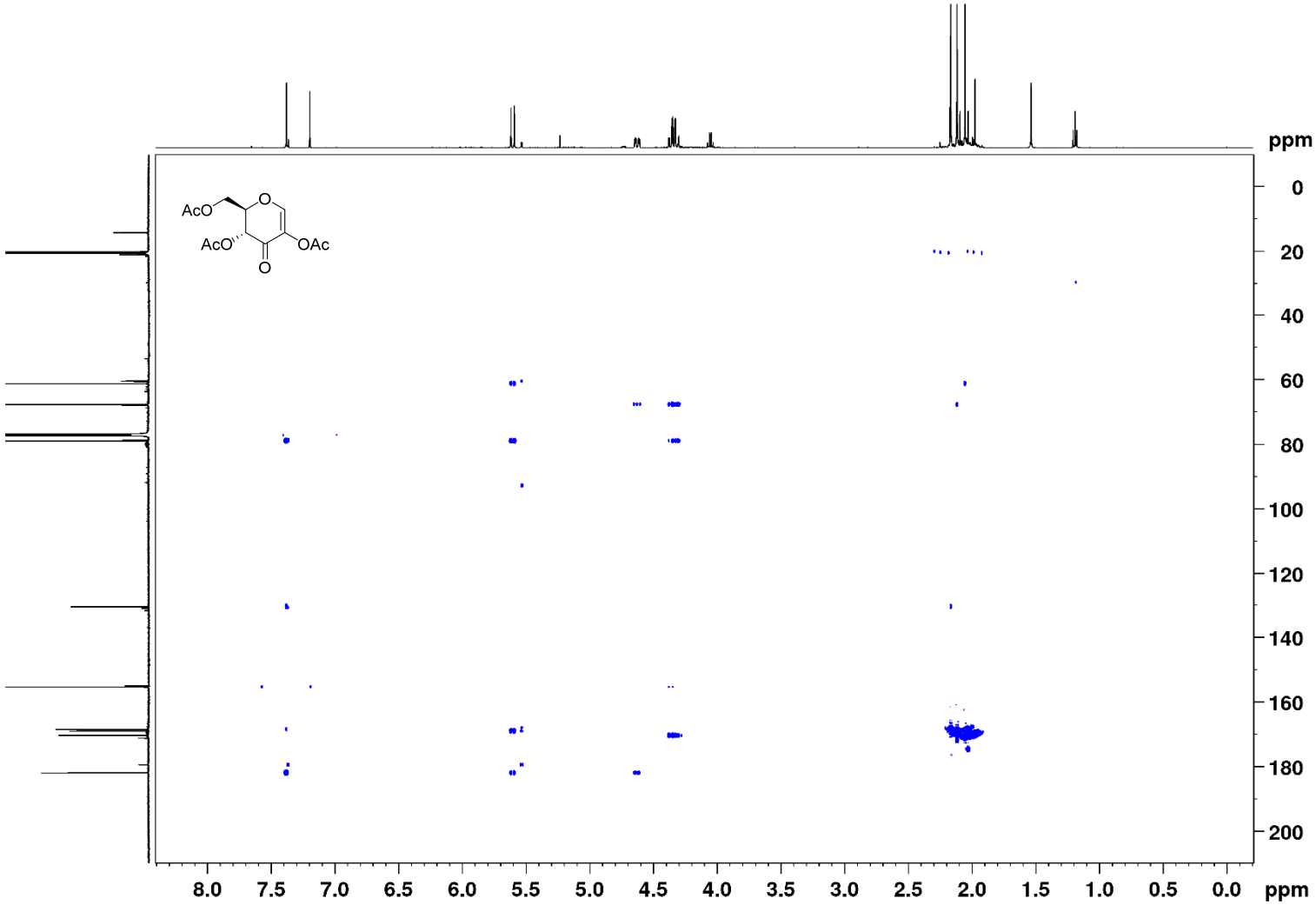
DQF-COSY NMR ($CDCl_3$, 25 °C):



HSQC NMR ($CDCl_3$, 25 °C):

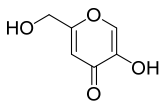


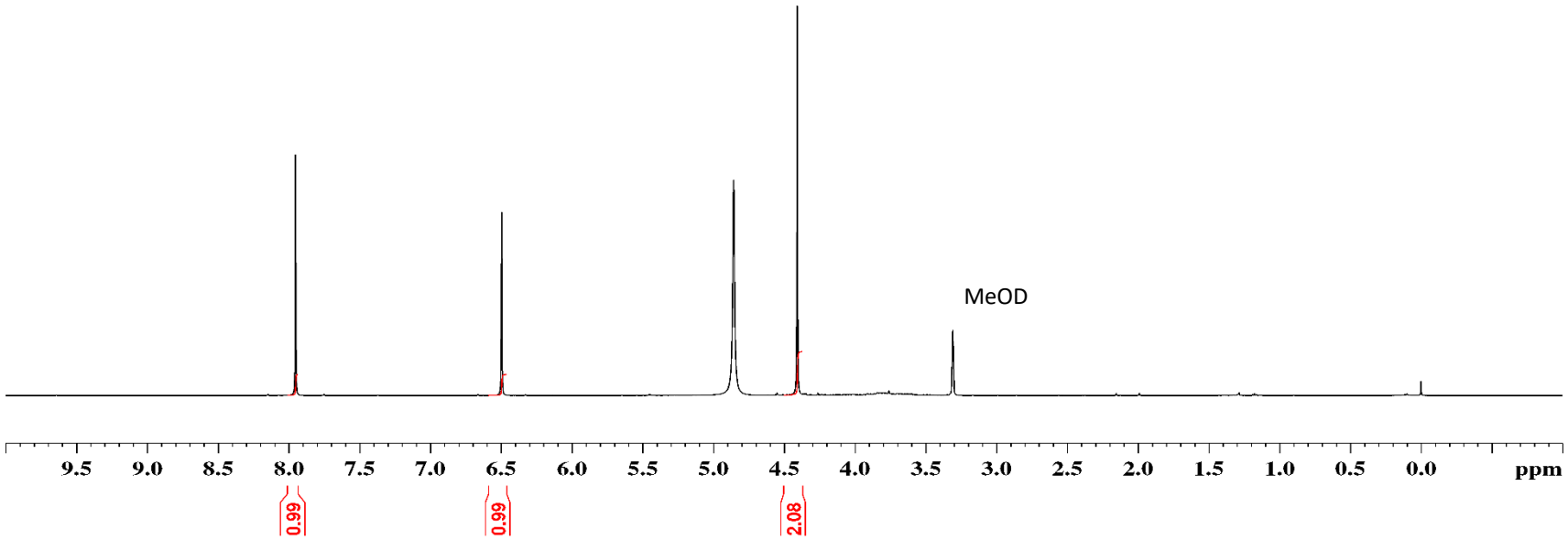
HMBC NMR ($CDCl_3$, 25 °C):



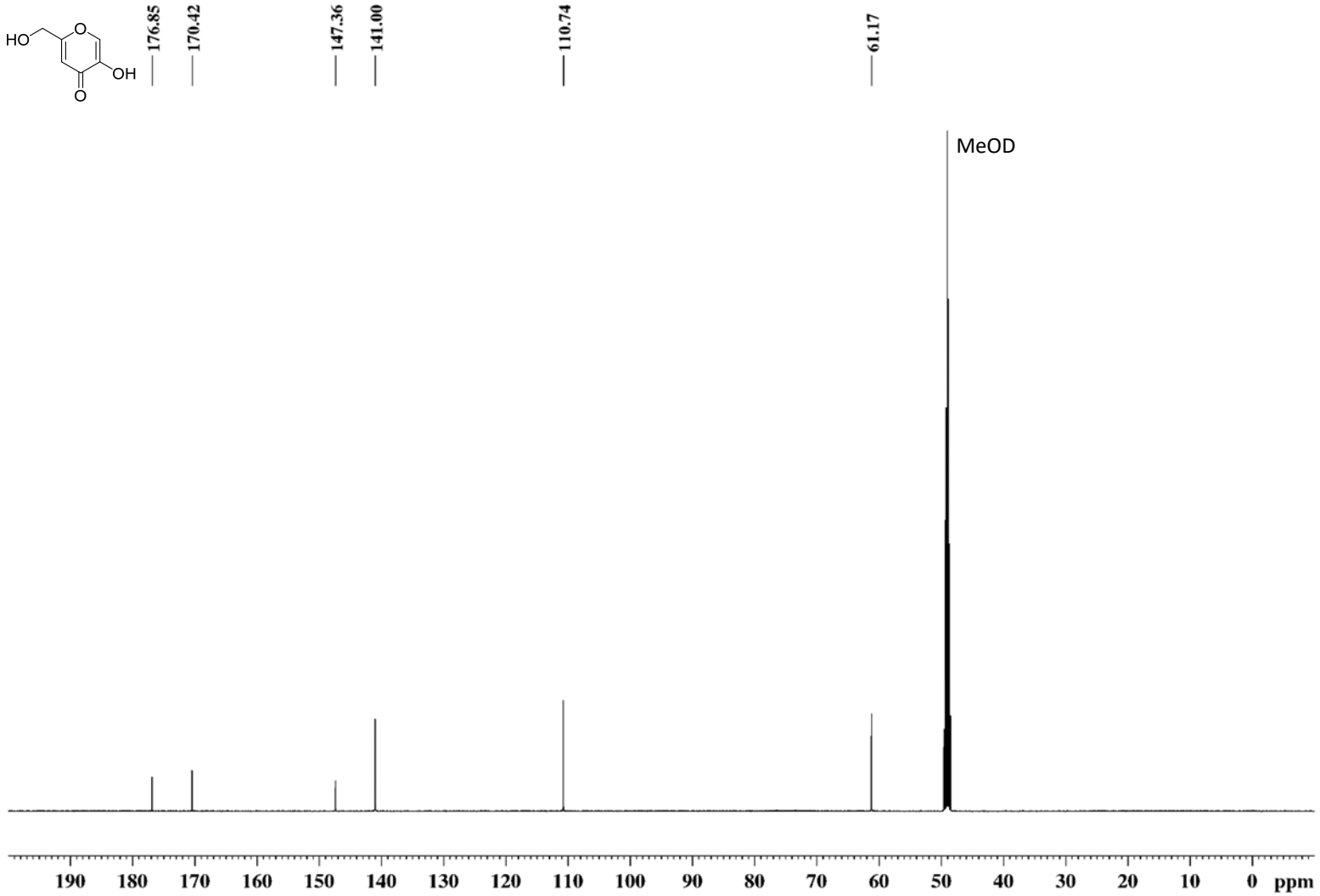
Kojic acid (1)

^{1}H NMR (500.20 MHz, MeOD, 25 °C):

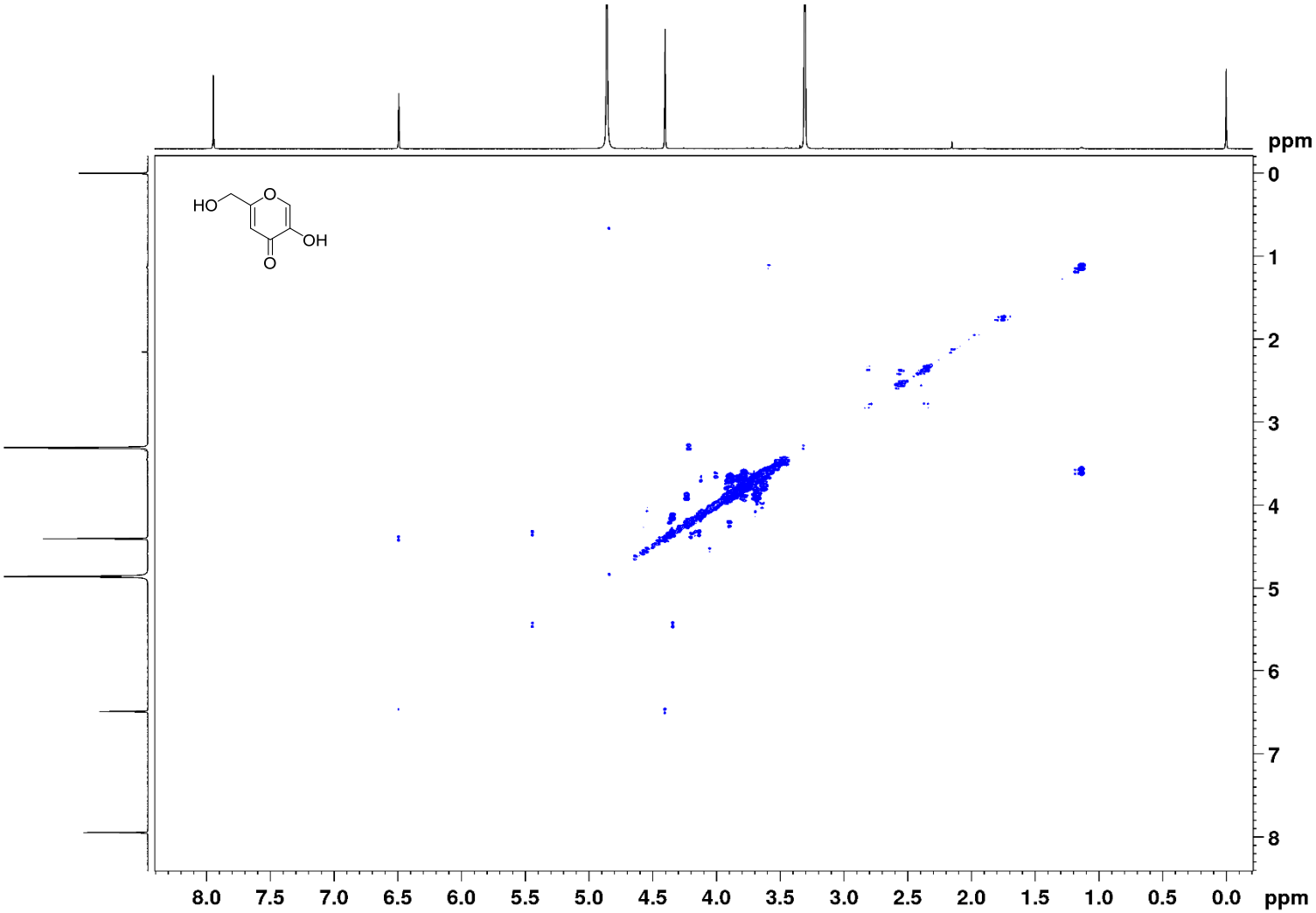




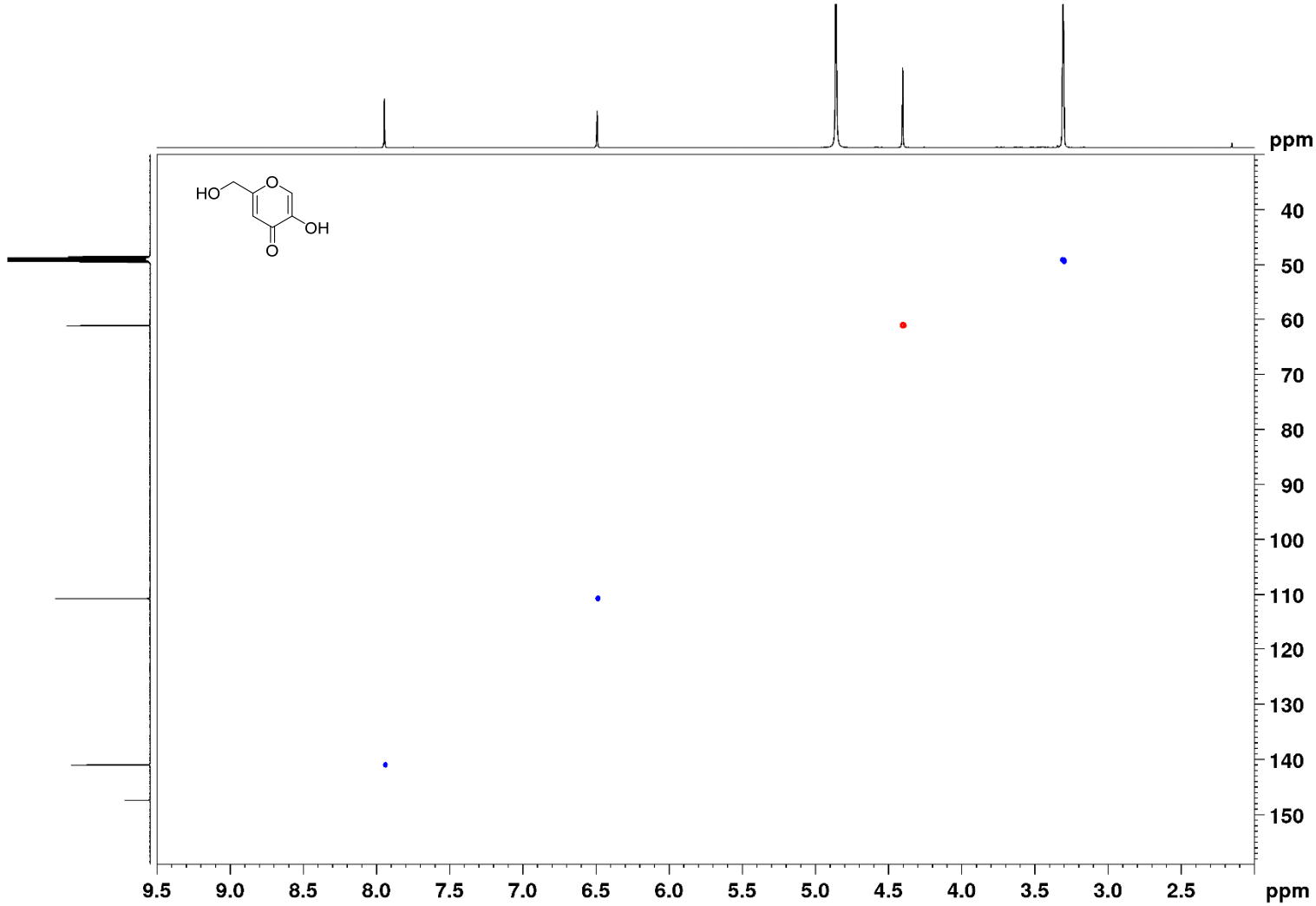
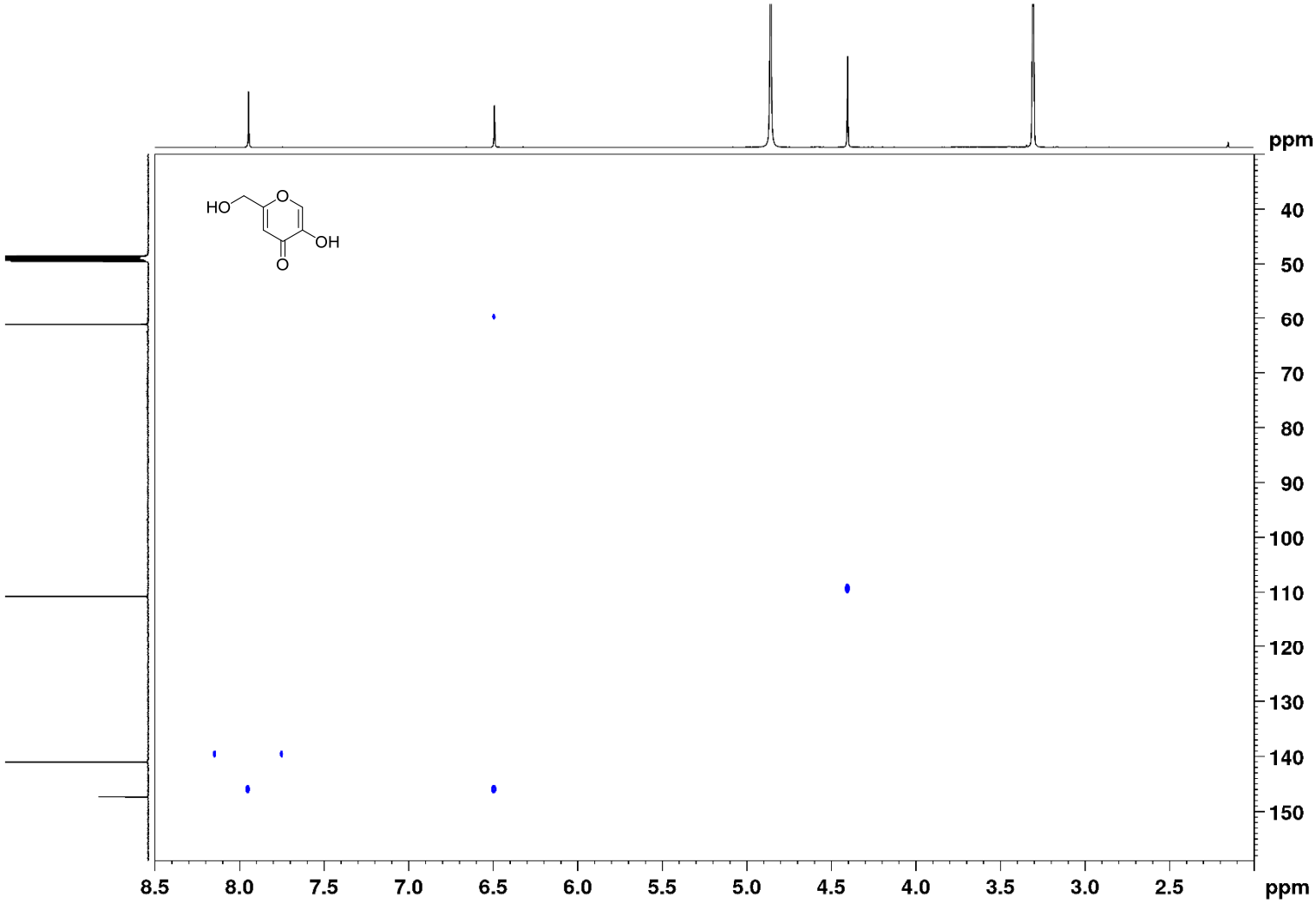
^{13}C NMR (125.8 MHz, MeOD, 25 °C):



DQF-COSY NMR (MeOD, 25 °C):



HSQC NMR (MeOD, 25 °C):



HMBC NMR (MeOD, 25 °C):