Electronic Supplementary Material (ESI) for Chemical Communications

A gram scale synthesis of a multi-^{13}C-labelled anthocyanin, [6,8,10,3′,5′-$^{13}C_5$]cyanidin-3-glucoside, for use in oral tracer studies in humans

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Experimental Methods

General information

NMR spectra were recorded on a Varian Gemini 2000 (^{1}H 300 MHz, ^{13}C 75.45 MHz) or a Bruker Advance 400 (^{1}H 400 MHz, ^{13}C 100.16 MHz) spectrometer. Chemical shifts (δ) in ppm are given relative to Me_4Si, coupling constants (J) are given in Hz. IR spectra were recorded on a Perkin-Elmer 2000 FTIR spectrometer as KBr disc. Low resolution and high resolution electrospray mass spectra were recorded on a Micromass LC-T (time-of-flight). Melting points were recorded on an Electrothermal melting point apparatus and are uncorrected. Analytical TLC was carried out on either Merck Silica 60 F_{254} or Cellulose F or RP-18 F_{254S} plates. The components were observed under ultraviolet light (254 nm). [1,3-$^{13}C_2$]Acetone and diethyl [2-^{13}C]malonate were purchased from Sigma Aldrich. Diethyl [2-^{13}C]malonate was dried over 4Å molecular sieve (flamed dried in advance) and distilled or dried over 4Å molecular sieve through a Soxhlet apparatus using anhydrous ethanol as azotropic refluxing solvent. Anhydrous ethanol, *tert*-butanol, dichloromethane and acetonitrile were obtained by drying and distillation over calcium hydride and stored under argon. EtOAc was dried over calcium hydride. Other chemicals were used as delivered from Sigma-Aldrich or Alfa Aesar unless otherwise stated. All reactions were carried out in batches in the typical scales as described below under an argon atmosphere or in glassware flushed with argon in advance.

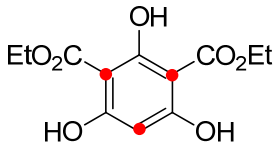
All labelled compounds show the expected coupling pattern in their ^{1}H NMR and ^{13}C NMR. The enhanced peaks of the labelled carbons suppress the signals of other carbons so much that some of them, in particular the tertiary carbons coupled with the labelled one(s), are invisible in their ^{13}C NMR spectra. Only the observed resonances were listed.

HPLC analyses

HPLC analyses for the final reaction mixture and isolated fractions were carried out on a Waters HPLC system (Waters 600 pump), Waters 2700 Sample manager, Waters 2487 dual wavelengths absorbance detector. The HPLC system was operated using a Phenomenex Kingsorb C-18 analytical column (250 × 4.6 mm, 3 µm) equipped with a security guard cartridge. Mobile phase consisted of 5% formic acid in water (A) and 5% formic acid in acetonitrile (B). The program ran a linear gradient from 2 to 25% B in 15 min, 45% B in 20min and 100% B in 30 min and 2% B in 35 min followed by isocratic conditions with 2% B for 5 min to equilibrate the column. Peaks were detected at 525 nm at a flow rate of 1.2 mL/min.

The purity of [$^{13}C_5$]C3G **1** was analysised on a system optimised exclusively for anthocyanines. Column: Eclipse XDB-C18 4.6 × 150 uM; Mobile phase: 0.5% formic acid in water (A) and 0.5% formic acid in MeOH (B); Gradient: 2% B at 0 min, 20% B in 4 min, 80% B in 6 min, 90% B in 8 min, 100% B in 10–14 min, 0.5% B in 15-18 min; Flow rate: 1 ml/min; Injection volumn: 5.0 µl; Sample concentration: 50 µM of [$^{13}C_5$]C3G **1** in 0.01% DMSO in 0.01% formic acid vv 100% methanol. Wavelength: 525 nm.

Diethyl 2,4,6-trihydroxy-[1,3,5-$^{13}C_3$]isophthalate **4**. [1]



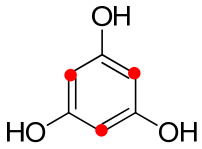
4

Diethyl [2-^{13}C]malonate (31.5 g, 195 mmol) was added to a freshly prepared solution (63 mL) of sodium ethoxide prepared by the addition of sodium metal (2.58 g, 112 mmol) into anhydrous EtOH (63 mL). The mixture was heated at 135-138°C for 48 h, cooled and acidified with 15% of HCl. The solid precipitate was filtered, washed with cold EtOH and Et_2O and dried at 40°C for 16 h to give the product as off-white solid. Yield: 4.8 g, 27%. m. p. 101-103°C (unlabelled counterpart 108-109°C, Lit. for unlabelled[2] 106-107 °C). ν_{max} (KBr disc) 3340 (OH), 1646 (C=O), 1577 (Ar C=C), 1249, 1166 (CO_2Et); δ_H (400 MHz, $CDCl_3$) 11.84 (br s, 1H, OH), 11.30 (v br s, 2H,

2 × OH), 6.06 (dt, 1H, $^{1}J_{CH}$ 166.6 Hz, $^{3}J_{CH}$ 5.4 Hz, H-5); 4.51 (q, 4H, $^{3}J_{HH}$ 7.2 Hz, 2 × CH_2), 1.46 (t, 6H, *J* 7.2 Hz, 2 × CH_3); δ_C (100.16 MHz, $CDCl_3$) 97.0 (t enhanced, $^{2}J_{CC}$ 3.3 Hz, ^{13}C-5), 94.8 (d enhanced, $^{2}J_{CC}$ 3.0 Hz, ^{13}C-1 and ^{13}C-3), 62.6, 14.5, other carbon signals are suppressed. *m/z* (ES^+) 296 $[M + Na]^+$; HRMS (ES^+) 296.0748, $^{13}C_3C_9H_{14}O_7Na$ requires 296.0738.

After collection of the product, the filtrate was saturated with sodium chloride and extracted into EtOAc (three times). The combined extracts were dried (Na_2SO_4) and the solvent removed. The opaque oil was diluted with diethyl ether and filtered to remove any solid particulate. The filtrate was evaporated to recover unreacted starting material (6.9 g, recovery rate 22%) as a brown oil, which could then be recycled.

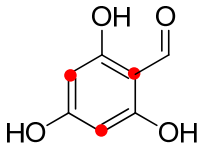
1,3,5-Trihydroxy-[2,4,6-$^{13}C_3$]benzene **5**.[1]



5

Diethyl 2,4,6-trihydroxy-[1,3,5-$^{13}C_3$]isophthalate **4** (6.0 g, 21.96 mmol) was added to 60% aqueous potassium hydroxide (24.5 g of potassium hydroxide in 17 mL of water) solution. The mixture was heated at 130°C for 2 h. After cooling to RT, the mixture was acidified with ice cold aq. HCl (15%). The pinkish solid precipitate was collected by filtration and washed sequentially with water, cold ethanol and cold ether and then dried to give the product (2.7 g, 96%). An aliquot was further purified by chromatography (SiO_2, EtOAc : Petroleum ether = 2 : 1) for analysis. m. p. 195-197 °C (Lit.[3] for unlabelled 218-221°C); ν_{max} (cm^{-1}, KBr disc) 3273 (OH), 1603 (aromatic C=C), 1493 (aromatic C=C); δ_H (300 MHz, MeOD) 5.78 (dt, 3H, $^{1}J_{CH}$ 158.7 Hz, $^{3}J_{CH}$ 4.6 Hz, H-2, H-4 and H-6); δ_C (75.45 MHz, MeOD) 95.6 (s enhanced, ^{13}C-2, ^{13}C-4 and ^{13}C-6); *m/z* (ES^-) $[M - H]^-$ 128; HRMS (ES^-) 128.0338, $^{13}C_3C_3H_5O_3$ requires 128.0339.

2,4,6-Trihydroxy-[1,3,5-$^{13}C_3$]benzaldehyde **6**.



6

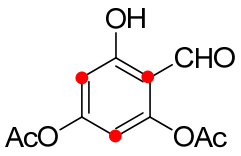
2,4,6-Trihydroxy-[1,3,5-$^{13}C_3$]benzaldehyde **6** was prepared by a Vilsmeier-Haack reaction[4] using oxalyl chloride in place of $POCl_3$. A solution of oxalyl chloride (2.4 mL, 27.8 mmol) in acetonitrile (20 mL) was added to a solution of anhydrous DMF (2.7 mL, 34.9 mmol) in acetonitrile (60 mL), dropwise over 10 min at room temperature. Gas evolved and a precipitate formed. The reaction was stirred at ambient temperature for 1 h to ensure complete conversion of the oxalyl chloride to the Vilsmeier iminium cation. The reaction mixture was then cooled in a dry-ice bath to -40°C and a solution of 1,3,5-trihydroxy-[2,4,6-$^{13}C_3$]benzene (3. 0 g, 23.3 mmol) in acetonitrile was added through a cannular. A sticky gum formed immediately. After vigorous stirring and swirling for 20 min at -20°C, the sticky gum gradually solidified. Stirring was continued for another 30 min at this temperature and then the solution was allowed to warm up to ambient temperature and was stirred for 2 h. Filtration followed by washing with cold acetonitrile and hexane gave the imine chloride salt as a pink solid.

The imine chloride salt: 5.0 g, 97%. M.p. >165°C (decomp). δ_H (300 MHz, DMSO-d_6) 11.32 (s, 1H, OH), 11.29 (s, 1H, OH), 10.77 (br 1H, OH), 8.78 (br s, 1H, CH=), 6.09 (m, 5.78 (dt, 3H, $^1J_{CH}$ 166.8 Hz, $^3J_{CH}$ 5.2 Hz, H-3 and H-5), 3.71 (s, 3H, CH_3), 3.34 (s, 3H, CH_3); δ_C (75.47 MHz, DMSO-d_6) 97.3 (t enhanced, $^2J_{CH}$ 3.5 Hz, ^{13}C-1), 94.7 (d enhanced, $^2J_{CH}$ 3.9 Hz, ^{13}C-3 and ^{13}C-5), 48.8 (CH_3), 45.1 (CH_3).

The salt was then dissolved in water (100 mL) and heated at 70°C for 30 min. Once cooled to 35°C, sodium thiosulfate solution (90 mM, 1.5 mL) was added to remove the resulting pink color. The mixture was then cooled to 5°C and the precipitate was filtered, washed with cold water and air dried to give the product as a pink solid (2.8 g, 77%). Due to difficult stirring in the process of imine chloride formation some batches (especially at a 6 g scale of [2,4,6-$^{13}C_3$]trihydroxybenzene) were contaminated with the diformylated product as indicated by ^{1}H NMR. Where diformylation had occured, the product was purified by column chromatography

(SiO_2, DCM : EtOAc 4 : 1 to 1 : 1). Analytical data is reported for the purified 2,4,6-trihydroxy-[1,3,5-$^{13}C_3$]benzaldehyde **6**. m.p. >210°C (dec.) [Sigma-Aldrich for unlabelled >195°C (dec.)]; ν_{max} (cm^{-1}, KBr disc) 3550 (OH), 3329 (br s, OH), 1626 (C=O, hydrogen bonded), 1590 (C=C). δ_H (400 MHz, MeOD) 10.01 (d, 1H, $^2J_{CH}$ 17.3 Hz, CHO), 5.77(m, 2H, $^1J_{CH}$ 161.0 Hz, $^3J_{CH}$ 5.1 Hz, H-3 and H-5); δ_C (75.45 MHz, MeOD) 106.4 (t, enhanced, $^2J_{CC}$ 2.8 Hz, ^{13}C-1), 95.3 (d, enhanced, $^2J_{CC}$ 2.8 Hz, ^{13}C-3 and ^{13}C-5); *m/z* (ES$^-$) 156 [M - H]$^-$; HRMS (ES$^-$) 156.0284, $^{13}C_3C_4H_5O_4$ requires 156.0288.

2,4-Diacetoxy-6-hydroxy-[2,4,6-$^{13}C_3$]benzaldehyde **2**. ^{13}

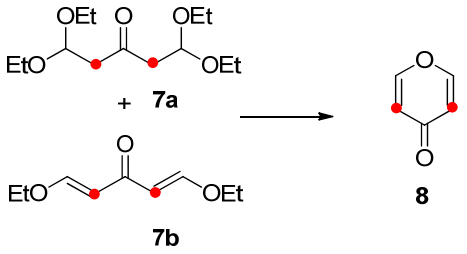


2

Acetic anhydride (9.50 mL, 100.7 mmol) was added to a solution of **6** (7.9 g, 50.3 mmol) in anhydrous ethyl acetate (300 mL) and then 4-dimethylaminopyridine (0.7 g (5.74 mmol) was added. The mixture was heated under reflux for 17 h before being cooled to RT. After passing through a pad of celite, the filtrate was washed with sat. bicarbonate solution. The aqueous layer was extracted into EtOAc (2 × 300 mL) and the organic layers were combined and dried ($MgSO_4$). The product was purified by chromatography (SiO_2, EtOAc ; petroleum ether 1: 3 to 1: 2) and the resultant waxy residue was recrystalized from acetonitrile/diethyl ether to give the product as a white solid (4.73 g, 39%). M.p. 93-95°C (Lit. for unlabelled[5] 93-95°C). ν_{max} (cm^{-1}, KBr disc) 3108 (OH), 1775 (C=O), 1651 (C=O), 1605 (aromatic C=C), 1560 (C=C), 1208, 1180, 1128 (COO); δ_H (400 MHz, $CDCl_3$) 11.78 (dd, 1H, $^3J_{CH}$ 7.6, 4.9 Hz, OH), 10.04 (m, 1H, $^2J_{CH}$ 19.2, $^3J_{CH}$ 2.0 Hz, CHO), 6.66 (ddt, $^1J_{CH}$ 167.6, $^3J_{CH}$ 5.0, $^4J_{HH}$ 2.1 Hz, H-3), 6.62 (m, 1H, $^1J_{CH}$ 170.5, $^3J_{CH}$ 4.6, $^4J_{HH}$ 2.1 Hz, H-5), 2.38, 2.37 (2 × s, 2 × 3H, 2 × Ac). δ_C (75.45 MHz, $CDCl_3$) 192.1 (d, $^1J_{CC}$ 56.8 Hz, CHO), 168.4 (C=O of Ac), 168.1 (C=O of Ac), 111.4 (t, enhanced , $^2J_{CC}$ 2.0 Hz, ^{13}C-1), 108.5 (br s, enhanced , ^{13}C-3), 107.9 (br s, enhanced , ^{13}C-5), 21.4 (s, CH_3 of Ac), 21.0 (s, CH_3 of Ac). *m/z*

(ES$^+$) 264 [M + Na]$^+$, 505 [2M + Na]; (HRESIMS$^+$) 264.0473 [M + Na]$^+$; $^{13}C_3C_8H_{10}O_6Na$ requires 264.0476.

4*H*-[3,5-$^{13}C_2$]Pyran-4-one **8**. [6,7]



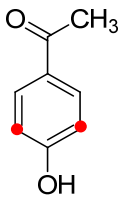
A solution of $BF_3.OEt_2$ (117.0 g, 100 mL, 824 mmol) in DCM (100 mL) was added dropwise over 60 min to triethylorthoformate (40 g, 43.8 mL, 263 mmol) at -40 to -30°C. The mixture was warmed to 0°C and kept at this temperature for 30 min. The mixture was then cooled to -78°C and [1,3-$^{13}C_2$]acetone (7.8 g, 9.5 mL, 130 mmol) was added. Diisopropylethylamine (107 g, 143 mL, 824 mmol) was then added dropwise over 1.5 h with stirring at the very beginning and swirling later as the mixture became very sticky. The resulting mixture was kept at -78°C for 3 h with swirling before being poured into aq sodium bicarbonate solution (200 g dissolved in 2 L of water). The mixture was stirred vigorously until no more gas evolved and was then extracted into dichloromethane (3 × 800 mL). The combined organic layers were washed successively with ice cold 1M H_2SO_4 (850 mL), cold water (2 × 1.2 L) and brine (1.2 L) and then dried ($MgSO_4$). Removal of solvent at reduced pressure gave a dark red oil, which was a mixture of 1,1,5,5-tetraethoxy-[2,4-$^{13}C_2$]pentan-3-one **7a** and (1*E*, 4*E*)-1,5-diethoxy-[2,4-$^{13}C_2$]penta-1,4-dien-3-one **7b** in the ratio of *ca.* 55 : 45 according to ^{1}H NMR spectrum. The crude mixture was not separated but used directly in the next reaction.

1,1,5,5-Tetraethoxy-[2,4-$^{13}C_2$]pentan-3-one **7a**. δ_H (400 MHz, $CDCl_3$) 4.91 (t, 2H, $^3J_{HH}$ 5.5 Hz, 2 × CH), 3.70-3.63 (m, 2H, C*H*$_2$CH$_3$, $^3J_{HH}$ 7.1 Hz), 3.57-3.50 (m, 2H, C*H*$_2$CH$_3$, $^3J_{HH}$ 7.1 Hz), 2.79 (ddd, 4H, ^{13}CH$_2$, $^1J_{CH}$ 128.1 Hz, $^3J_{CH}$ = $^3J_{HH}$ 5.6 Hz).

(*1E, 4E*)-1,5-Diethoxy-[2,4-$^{13}C_2$]penta-1,4-dien-3-one **7b**. δ_H (400 MHz, $CDCl_3$) 7.59-7.53 (m, 2H, H-1 and H-5), 5.60 (m, 2H, $^1J_{CH}$ 158.7 Hz, H-2 and H-4), 3.91 (q, 4H, $^3J_{HH}$ 7.1 Hz, 2 × CH_2), 1.31 (t, 6H, $^3J_{HH}$ 7.1 Hz, 2 × CH_3).

The dark oil (**7a** + **7b**) obtained above was dissolved in ethanol (500 mL) and 10% aqueous hydrochloric acid (50 mL). The mixture was heated at 80°C for 24h before solvent was removed under reduced pressure. The resulting residue was dissolved in water (500 mL) and washed with toluene (500 mL) and the toluene layer was then washed with water (2 × 500 mL). The combined aqueous layers was decoloured using actived charcoal and filtered through a bed of celite. Removal of water at reduced pressure gave 4*H*-[2,6-$^{13}C_2$]pyran-4-one **2** as a brown semi-solid. The semi-solid was then dissolved in minimum volume of EtOH and the solution was triturated with Et_2O. The off-white solid (10.2 g, 80%) that precipitated was filtered, washed with Et_2O, dried and used for analysis. In other batches, the reaction mixture was simply decolored by activated carbon and filtered through a pad of celite. Removal of the solvents under reduced pressure gave the product (quantitative, solid or semi-solid) which could be used for next step of reaction without further purification. m. p. 31-33°C (Sigma-Aldrich catalogue for unlabelled 32-34°C); (ν_{max} (KBr disc) 1601 (C=O), 1495 (C=C); δ_H (300 MHz, MeOD) 8.13 (m, 2H, H-2 and H-6), 6.30 (m, 2H, $^1J_{CH}$ 171.7 Hz, H-3 and H-5); δ_C (75.45 MHz, DMSO-d_6) 117.5 (s, enhanced, ^{13}C-3, ^{13}C-5), 156.9 (d, $^2J_{CC}$ 68.9 Hz, C-2, C-4), C=O not observed due to the suppression from the enhanced the signal; *m/z* (ES^+) 99 $[M + H]^+$, 121 $[M + Na]^+$; HRMS (ES^+) 121.0174, $^{13}C_2C_3H_4O_2Na$ requires 121.0176.

4-Hydroxy-[3,5-$^{13}C_2$]acetophenone **9**. [8]

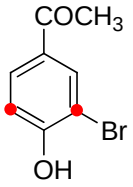


9

1M potassium *tert*-butoxide solution (freshly made from dissolving 10.0 g potassium metal in 256 mL of dry *tert*-butanol) was added to a refluxing solution of **8** (13.5 g, 138 mmol) and acetylacetone (35 g, 349 mmol) in dry *tert*-butanol (500 mL). The

reaction was kept at reflux for 24 h before 2 M HCl (165mL) was added. The acidified mixture was refluxed for another 2 h and then the *tert*-butanol was removed at reduced pressure. The aqueous residue was extracted into EtOAc (3 × 200 mL) and the combined extracts were washed with water (× 2) and brine and dried ($MgSO_4$). Solvent removal gave the product as deep red liquid which was subject to column chromatography (SiO_2, Petroleum ether : EtOAc = 4 : 1 to 1 : 1). The fractions containing the product were combined and solvent removed to give a dark solid. Recrystallization from DCM led to the product as an off-white solid (9.0 g, 47%). m. p.107-109°C, (Lit. for unlabelled[9] 106-107°C); ν_{max} (KBr disc) 3309 (OH), 1660 (C=O), 1587, 1564 (Ar C=C); δ_H (300 MHz, $CDCl_3$) 8.12 (br s, 1H, OH), 7.93 (d, 2H, $^3J_{HH}$ 8.8 Hz, H-2 and H-6), 6.47 (m, 2H, $^1J_{CH}$ 165.5, $^3J_{HH}$ 8.8 Hz, H-3 and H-5); δ_C (100.16 MHz, , $CDCl_3$) 199.1 (br s, C=O), 161.2 (t, $^2J_{CC}$ 65.0 Hz, C-4), 131.5 (dd, $^2J_{CC}$ 59.1, $^3J_{CC}$ 5.2 Hz, C-2, C-6), 129.6 (br s, C-1), 115.8 (s enhanced, ^{13}C-3 and ^{13}C-5), 26.50 (CH_3). *m/z* (ES^+) 139 $[M + H]^+$, 161 $[M + Na]^+$; (ES^-) 137 $[M - H^+]$; HRMS (ES^+) 161.0490, $^{13}C_2C_6H_8O_2Na$ requires 161.0489.

3-Bromo-4-hydroxy-[3,5-$^{13}C_2$]acetophenone **10**.

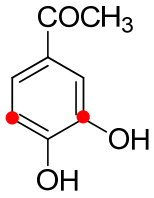


10

Bromine (2.2 mL, 42.82 mmol) was added dropwise to a solution of 4-[3,5-$^{13}C_2$]hydroxyacetonphone (5.0 g, 36.16 mmol) in DCM (180 mL) at 0°C. The mixture was stirred at 0°C for 40 min before ice cold water (180 mL) was added. The aqueous layer was extracted with three portions of DCM (200 mL) and the combined extracts were washed with saturated sodium metabisulfite (×2) and brine successively and dried ($MgSO_4$). The solvent was removed to give the product which contained *ca.* ~76% mol of the expected product **10**, *ca.* 13% mol of 3,5-dibromo-4-hydroxy-[3,5-$^{13}C_2$]acetophenone and *ca.*10% mol of starting material **9**. An aliquot of this product was purified by column chromatography (SiO_2, DCM) for analysis. The rest was

treated directly without further purification. Purified **10**. m.p. 103-105°C (unlabelled 104-106°C, Lit. for unlabelled[10] 108-110°C); ν_{max} (KBr disc) 3081 (OH), 1651 (C=O), 1581, 1547 (Ar C=C); δ_H (300 MHz, $CDCl_3$) 8.14 (ddd, 1H, $^2J_{CH}$ 4.2, $^4J_{CH}$ 1.2, $^4J_{HH}$ 2.1 Hz, H-2), 7.86 (m, 1H, $^3J_{HH}$ 8.5 Hz, H-6), 7.08 (ddd, 1H, $^1J_{CH}$ 164.1, $^3J_{CH}$ 7.8, $^3J_{HH}$ 8.5 Hz, H-5), 6.17 (dd, 1H, $^2J_{CH}$ 7.3, $^4J_{CH}$ 3.8 Hz, OH), 2.56 (s, 3H, CH_3); δ_C (75.12 MHz, , $CDCl_3$) 196.9 (C=O), 159.9 (very weak, C-4), 133.2 (dd, $^1J_{CC}$ 67.7, $^3J_{CC}$ 5.4 Hz, C-2), 131.7 (s, C-1), 130.2 (dd, $^1J_{CC}$ 59.8, $^3J_{CC}$ 6.3 Hz, C-6), 116.1 (d enhanced, $^2J_{CC}$ 5.5 Hz, ^{13}C-5), 11.8 (d enhanced, $^2J_{CC}$ 5.5 Hz, ^{13}C-3), 26.5 ($\underline{C}H_3$). *m/z* (ES^-) 214.9/216.9 [$M\text{-}H^+$]; HRMS (ES^-) 214.9619/216.9607, $^{13}C_2C_6H_6{}^{79}BrO_2$ / $^{13}C_2C_6H_6{}^{81}BrO_2$ requires 214.9618/216.9598.

3,4-Dihydroxy-[3,5-$^{13}C_2$]acetophenone **11**[7]

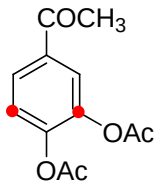


11

$CuSO_4 \cdot 5H_2O$ (9.78 g, 4 mmol) was added to a solution of sodium hydroxide (155 g, 3.875 mol) in water (1000 mL) and the reaction was stirred under reduced pressure for 4 h. The resulting suspension was transferred by cannula to a flask containing cr **10** (20 g, *ca.* 92.2 mmol). The mixture was heated at reflux for 24 h, cooled and acidified with conc. HCl. The aqueous mixture was extracted into EtOAc (800 mL × 6), the combined extracts were dried ($MgSO_4$) and the solvent removed at reduced pressure to yield a dark red solid which contained *ca.* 88% mol of **11**, *ca.* 10% mol of starting material **9** and a small amount of 3,4,5-trihydroxy-[3,5-$^{13}C_2$]acetophenone based on ^{1}H NMR analysis. An aliquot of this product was subject to chromatography to provide a sample of **11** for characterization. m. p. 115-117°C (Lit. for unlabelled[11] 117-119°C. ν_{max} (KBr disc, cm^{-1}) 3370 (OH), 1665 (C=O), 1573 (Ar C=C); δ_H (300 MHz, MeOD) 7.45-7.40 (m, 2H, H-2, H-6); 6.82 (ddd, 1H, $^1J_{CH}$ 159.2, $^3J_{CH}$ 7.0, $^3J_{HH}$ 8.5 Hz, H-5), 2.50 (s, 3H, CH_3); δ_C (100.16 MHz, MeOD) 199.9 (t, $^3J_{CC}$ 5.2 Hz, $COCH_3$), C-4 not observed, 146.5 (d enhanced, $^2J_{CC}$ 6.5 Hz, ^{13}C-3), 130.8

(s, C-1), 123.7 (dd, $^{1}J_{CC}$ 58.6, 5.8 Hz, C-6), C-2 overlapped by C-5, 115.9 (d enhanced, $^{2}J_{CC}$ 6.1 Hz, ^{13}C-5), 26.4 (CH_3). *m/z* (ES^-) 153 $[M-H]^-$; (HRESIMS$^-$) 153.0461 $[M-H]^-$, $^{13}C_2C_6H_7O_3$ requires 153.0462. The majority of the crude product was used for the next reaction without further purification.

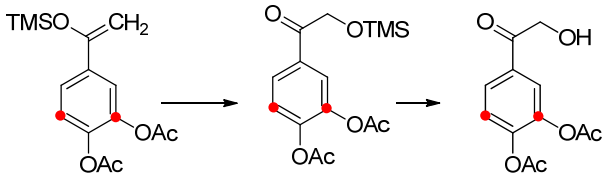
3,4-Diacetoxy-[3,5-$^{13}C_2$]acetophenone **12**.



12

A crude preparation of **11** (15.6 g, *ca* 97.2 mmol, resulted from 21.2 g of crude **10**) was suspended in EtOAc (220 mL) and acetic anhydride (40 mL, 424 mmol) and DMAP (3.66 g, 30 mmol) were added and the mixture was heated under N_2 at reflux for 44 h. After cooling to RT, the mixture was filtered though a pad of celite to remove any tar formed during the reaction. The filtrate was washed with saturated aq. sodium bicarbonate until no more gas evolved. The aqueous layer was extracted into EtOAc (×2) and the organic layers were combined, dried ($MgSO_4$) and the solvent removed. The residue was purified by chromatography to afford **11** as white flakes (14.0 g, *ca.* 50% over three steps from **9**). m. p. 79-81°C (Lit. for unlabelled[12] 84-85°C); ν_{max} (cm^{-1}, KBr disc) 1773 (C=O of ester), 1683 (C=O conjugated with the aromatic ring), 1215, 1200 (COO); δ_H (400 MHz, $CDCl_3$) 7.87 (m, 1H, $^{3}J_{HH}$ 8.4 Hz, H-6); 7.78 (m, 1H, $^{3}J_{HH}$ 2.0 Hz, H-2), 7.32 (ddd, 1H, $^{1}J_{CH}$ 165.9, $^{3}J_{CH}$ 8.0, $^{3}J_{HH}$ 8.0 Hz, H-5), 2.56 (s, 3H, CH_3), 2.33 (s, 3H, Ac), 2.327 (s, 3H, Ac); δ_C (75.47 MHz, $CDCl_3$) 196.1 (t, $^{3}J_{CC}$ 5.1 Hz, $COCH_3$), 168.2 (d, $^{2}J_{CC}$ 3.6 Hz, 3-CO of Ac), 167.9 (4-CO of Ac), C-4 very weak, 142.4 (d enhanced, $^{2}J_{CC}$ 5.3 Hz, ^{13}C-3), 135.8 (s, C-1), 127.3 (dd, $^{1}J_{CC}$ 58.0 Hz, $^{3}J_{CC}$ 5.1 Hz, C-6), C-2 overlapped by C-5, 123.8 (d enhanced, $^{2}J_{CC}$ 5.3 Hz, ^{13}C-5), 26.7 ($CO\underline{C}H_3$), 20.8 ($\underline{C}H_3$ of 4-OAc), 20.7 (d, $^{3}J_{CC}$ 2.0 Hz, $\underline{C}H_3$ of 3-OAc,); *m/z* (ES^+) 261 [M + Na]; HRMS (ES^+), 261.0648, $^{13}C_2C_{10}H_{12}O_5Na$ requires 261.0650.

α-Hydroxy-3,4-diacetoxy-[3,5-$^{13}C_2$]acetophenone **15**.[13,14]



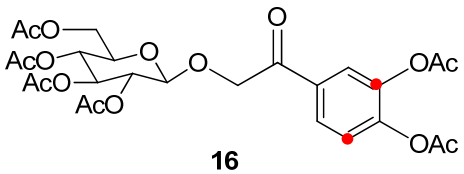
13 **14** **15**

Triethylamine (11.8 mL, 84.8 mmol) and trimethylsilyl chloride (6.6 mL, 52.0 mmol) were added to a solution of **12** (5.0 g, 20.99 mmol) in anhydrous DMF (5 mL) and the reaction was heated to 70°C for 24 h and then cooled. The mixture was diluted with ice-cold solvent (EtOAc : Petroleum ether = 1 : 2, 300 mL) and washed with ice-cold aqueous sat. sodium bicarbonate (200 mL). The organic layer was dried ($MgSO_4$) and the solvent removed to give silylenol ether **13** as an orange wax. δ_H (300 MHz, $CDCl_3$) 7.47(ddd, 1H, $^2J_{CH}$ 3.5, $^4J_{CH}$ 1.7, $^3J_{HH}$ 8.4 Hz, H-6); 7.38 (m, 1H, H-2), 7.16 (ddd, 1H, $^1J_{CH}$ 171.8, $^3J_{CH}$ 8.0, $^3J_{HH}$ 8.4 Hz, H-5), 4.88 (d, H, $^2J_{HH}$ 2.1 Hz, H of $=CH_2$), 4.43 (d, H, $^2J_{HH}$ 2.1 Hz, H of $=CH_2$), 2.31 (d, 3H, 3-OAc CH_3, $^4J_{CH}$ 0.4 Hz), 2.29 (s, 3H, 4-OAc CH_3), 0.29 (s, 9H, TMS). δ_C (75.47 MHz, $CDCl_3$) 142.0 (d enhanced, $^3J_{CC}$ 5.9 Hz, ^{13}C-3), 123.1 (d enhanced, $^3J_{CC}$ 5.8 Hz, ^{13}C-5).

α-Trimethylsilyloxy-3,4-diacetoxy-[3,5-$^{13}C_2$]acetophenone **14**. Anhydrous $MgSO_4$ was added to a solution of silylenol ether **13** in DCM (300 mL). The suspension was cooled to -20°C and *m*CPBA (<77%, 6.0 g, 29.9 mmol) was added portionwise. The mixture was stirred at this temperature for 5 h and then aqueous sodium sulphite solution was added and stirring continued for another 30 min. The resulting three-phase mixture was filtered and the residue washed several times with DCM. The filtrate was separated by layers. The organic layer was washed successively with sat. aq. sodium bicarbonate (× 3) and brine and dried ($MgSO_4$). Solvent removal afforded **14** as a white wax. δ_H (300 MHz, $CDCl_3$) 7.85 (m, 1H, $^3J_{HH}$ 8.4 Hz, H-6); 7.79 (m, 1H, H-2), 7.32 (ddd, 1H, $^1J_{CH}$ 166.0, $^3J_{CH}$ 7.7, $^3J_{HH}$ 8.4 Hz, H-5), 4.87 (s, 2H, CH_2), 2.33 (d, 3H, 3-OAc CH_3, $^4J_{CH}$ 0.44 Hz), 2.235 (s, 3H, 4-OAc CH_3); δ_C (75.47 MHz, $CDCl_3$) 142.5 (d enhanced, $^3J_{CC}$ 5.5 Hz, ^{13}C-3), 123.9 (d enhanced, $^3J_{CC}$ 5.5 Hz, ^{13}C-5).

α-Hydroxy-3,4-diacetoxy-[3,5-$^{13}C_2$]acetophenone **15**. A solution of product **14** in methanol (100 mL) was heated to reflux for 30 min. After removal of the solvent, the residue was purified by column chromatography (SiO_2, DCM : EtOAc = 8 : 1 to 4 : 1) and the recovered product was recrystallized from EtOAc and Et_2O to give a white solid (2.35 g, 44% over 3 steps). m. p. 90-92°C. (Lit. for unlabelled[15] 86-87°C); ν_{max} (KBr disc, cm^{-1}) 3535 (OH), 1761(Ac C=O), 1701 (C=O), 1251, 1203, 1149, 1090 (CO-O); δ_H (400 MHz, $CDCl_3$) 7.83 (m, 1H, $^3J_{HH}$ 7.8 Hz, H-6), 7.79 (ddd, $^2J_{CH}$ 4.6, $^4J_{CH}$ 0.9, $^4J_{HH}$ 2.1 Hz, H-2), 7.37 (ddd, 1H, $^1J_{CH}$ 166.4, $^3J_{CH}$ 8.3, $^3J_{HH}$ 7.7 Hz, H-5), 4.85 (d, 2H, $^3J_{HH}$ 4.7 Hz, CH_2), 3.41 (t, 1H, $^3J_{HH}$ 4.7 Hz, OH), 2.34 (d, 3H, $^3J_{CH}$ 0.3 Hz, 3-OAc), 2.33(s, 3H, 4-OAc). δ_C (75.47 MHz, $CDCl_3$) 196.8 (t,$^3J_{CC}$ 4.9 Hz, C=O), 168.1 (d, $^2J_{CC}$ 3.6 Hz, C=O of 3-OAc), 167.8 (s, C=O of 4-OAc), C-4 not observed, 142.8 (d enhanced, $^2J_{CC}$ 5.5 Hz, ^{13}C-3), 131.9 (s, C-1), 126.3 (dd, $^1J_{CC}$ 58.7, $^3J_{CC}$ 6.9 Hz, C-6), 124.4 (d enhanced, $^2J_{CC}$ 5.5 Hz, ^{13}C-5), 123.3 (dd, $^1J_{CC}$ 64.8, $^3J_{CC}$ 4.9 Hz, C-2), 65.7 (s, $\underline{C}H_2$), 20.9 (s, $\underline{C}H_3$ of 4-OAc), 20.8 (d, $^1J_{CC}$ 57.8, $^2J_{CC}$ 1.98 Hz, $\underline{C}H_3$ of 3-OAc). *m/z* (ES^+) 277 $[M + Na]^+$; ($HRESIMS^+$) 277.0591 $[M + Na]^+$; $^{13}C_2C_{10}H_{12}O_6Na$ requires 277.0599.

α-*O*-Tetraacetyl-ß-*D*-glucosidoxy-3,4-diacetoxy-[3,5-$^{13}C_2$]acetophenone **16**.[16]

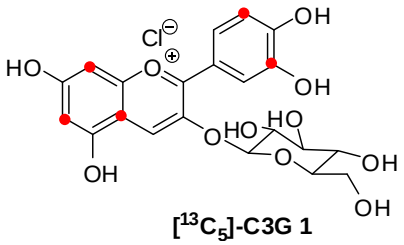


16

Powdered 4Å molecular sieve (2 g) were added to a solution of α-hydroxy-3,4-diacetoxy-[3,5-$^{13}C_2$]acetophenone (1.64 g, 6.44 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl trichloroacetimidate[16] (4.80 g, 9.74 mmol) in dry DCM (40 mL). The mixture was cooled to -20°C and $BF_3.Et_2O$ (0.4 mL, 3.24 mmol) was added dropwise. The mixture was allowed to warm gradually to 15°C and stirring was continued for 4 h. The reaction was quenched with triethylamine and the mixture was then filtered through a pad of celite and the filtrate was concentrated. The residue was purified by column chromatography (SiO_2, Petroleum ether: EtOAc 3 : 1 to 2.5: 2) and this gave **16** as white foam which could be powdered after drying under a high vacuum (1.8 g, 46%). m. p. 95-97°C, (Lit. for unlabelled[15] 105-105.5°C); $[\alpha]_D^{20}$ -28°

($CHCl_3$, c = 0.5), ν_{max} (KBr disc, cm^{-1}) 1760-1753 (C=O), 1701 (C=O), 1320, 1194, 1041 (COO); δ_H ($CDCl_3$, 400 MHz) 7.83 (m, 1H, $^3J_{HH}$ 8.0 Hz, aromatic H-6), 7.78 (m, 1H, $^2J_{CH}$ 4.67, $^4J_{CH}$ 1.0, $^4J_{HH}$ 2.0 Hz, aromatic H-2), 7.38 (ddd, $^1J_{CH}$ 166.4, $^3J_{CH}$ 7.8, $^3J_{HH}$ 8.0 Hz, aromatic H-5), 5.24 (dd, $^3J_{HH}$ 9.6, 9.5 Hz, Glu H-3), 5.12-5.07 (m, 2H, Glu H-2 + H-4), 4.90, 4.86 (2 × d, 2 H, AB, $^2J_{HH}$ 16.3 Hz, $COCH_2O$), 4.67 (d, 1H, $^3J_{HH}$ 7.8 Hz, Glu H-1), 4.24, 4.23 (dd, AB, 1H, $^2J_{HH}$ 12.3, $^3J_{HH}$ 4.6 Hz, Glu H-6a), 4.15-4.12 (m, 1H, Glu H-6b), 3.68 (ddd, $^3J_{HH}$ 10.0, 4.7, 2.2 Hz, Glu H-5), 2.333 (d, $^4J_{CH}$ 0.4 Hz, 3H, aromatic 3-OAc), 2.325 (s, 3H, aromatic 4-OAc), 2.09, 2.05, 2.03, 2.02 (4 × s, 12H, Glu 4 × OAc). δ_C (75.45 MHz, $CDCl_3$) 170.9, 170.4, 169.9, 169.6, 142.6 (d enhanced, $^2J_{CC}$ 5.5 Hz, aromatic ^{13}C-3), 124.1 (d enhanced, $^2J_{CC}$ 5.5 Hz, aromatic ^{13}C-5), 100.4, 72.6, 72.2, 71.1, 70.9, 68.4, 61.0, 20.9, 20.9, 20.84, 20.8 (overlapped). m/z (ES^+) 607 $[M + Na]^+$, ($HRESIMS^+$) 607.1536 $[M + Na]^+$, $^{13}C_2C_{24}H_{30}O_{15}Na$ requires 607.1550.

[6, 8, 10, 3′, 5′-$^{13}C_5$]Cyanidin-3-glucoside chloride [$^{13}C_5$]C3G **1** [13,15]



[$^{13}C_5$]-C3G 1

Hydrogen chloride was bubbled into a solution of **3** (1.02 g, 1.745 mmol) and **2** (0.42 g, 1.745 mmol) in dry ethyl acetate (22 mL) at -20°C. A deep red colour gradually developed. The mixture was then stirred at 4°C until all of the starting materials were consumed as monitored by TLC. The solvent was evaporated and the residue dissolved in acidic MeOH (0.1% HCl, 12 mL) and then aq. KOH (0.77 g, 13.7 mmol in 12 mL of water) was added. The mixture was stirred at RT for 3 h and then carefully acidified to pH 2.6 with 2N HCl. The solvents were removed under vacuum (40°C) and the residue was taken into acidic MeOH (0.1% HCl). The suspension was passed through a pad of celite to remove the majority of the precipitated KCl and then the filtrate was evaporated and the residue re-dissolved in water (0.1% HCl). The aqueous solution was then loaded onto Amberlite XAD-7 resin and eluted with a solvent gradient [from H_2O (0.1% HCl) to ethanol (0.1% HCl) :

H_2O (0.1% HCl) = 2 : 3]. The fractions were monitored variously by reverse phase TLC, cellulose TLC and by HPLC. The less pure fractions, after concentration, were further purified by cellulose microcrystalline (0.1% HCl) and C-18 reverse phase column chromatography [0.1% HCl : CH_3CN (+0.1% HCl) = 35 : 5]. The pure fractions were combined and evaporated in vacuum (40°C) and the residue was taken up in small amount of EtOH (0.1% HCl) and the mixture was sonicated. Trituration of the EtOH (0.1% HCl) solution with acetonitrile (0.1% HCl) led to precipitation of a dark red product. Filtration followed by washing with acetonitrile (0.1% HCl), diethyl ether and drying *in vacuo* afforded the product as a dark red solid (360 mg, 42%). m. p. 194°C (dec.) (unlabelled 192-194°C dec.) $[\alpha]_D^{20}$ could not be recorded due to the highly coloured solution (0.1% HCl, c = 0.3); ν_{max} (KBr disc, cm^{-1}) 3374 (OH), 1618 ($C=O^+$); δ_H (400 MHz, MeOD : CF_3CO_2D = 98 : 2) 9.03 (d, 1H, $^2J_{CH}$ 1.2 Hz, H-4), 8.25 (ddd, 1H, $^3J_{HH}$ 8.5, $^4J_{HH}$ 2.1, $^2J_{CH}$ 1.3 Hz, H-6′), 8.06 (dd, 1H, $^2J_{CH}$ 4.1, $^4J_{HH}$ 2.5 Hz, H-2′), 7.01 (ddd, 1H, $^1J_{CH}$ 161.7, $^2J_{CH}$ 7.0, $^3J_{HH}$ 8.6 Hz, H-5′), 6.87 (m, 1H, $^1J_{CH}$ 168.5 Hz, H-8, correlated with H-4), 6.67 (m, 1H, $^1J_{CH}$ 163.4 Hz, H-6), 5.30 (d, 1H, $^3J_{HH}$ 7.8 Hz, Glu-H-1), 3.92 (dd, 1H, $^2J_{HH}$ 12.1, $^3J_{HH}$ 2.2 Hz, Glu-H-6a), 3.72 (dd, 1H, $^2J_{HH}$ 12.3, $^3J_{HH}$ 6.0 Hz, Glu-H-6b), 3.68 (dd, 1H, $^3J_{HH}$ 9.0, 7.9 Hz, Glu-H-2), 3.58 (m, 1H, Glu-H-5), 3.55 (dd, 1H, $^3J_{HH}$ 8.8 Hz, Glu-H-3), 3.45 (dd, 1H, $^3J_{HH}$ 9.6, 8.9 Hz, Glu-H-4); δ_C (100.6 MHz, $CDCl_3$) 147.6 (d enhanced, $^3J_{CC}$ 6.4 Hz, ^{13}C-3′), 117.6 (d enhanced, $^2J_{CC}$ 6.4 Hz, ^{13}C-5′), 113.6 (br s enhanced, ^{13}C-10), 103.6 (br s enhanced, ^{13}C-8), 95.3 (br s enhanced, ^{13}C-6). *m/z* (ES^+) 454 $[M-Cl]^+$, ($HRESIMS^+$) 454.1235 $[M-Cl]^+$; $^{13}C_5C_{16}H_{21}O_{11}$ requires 454.1252.

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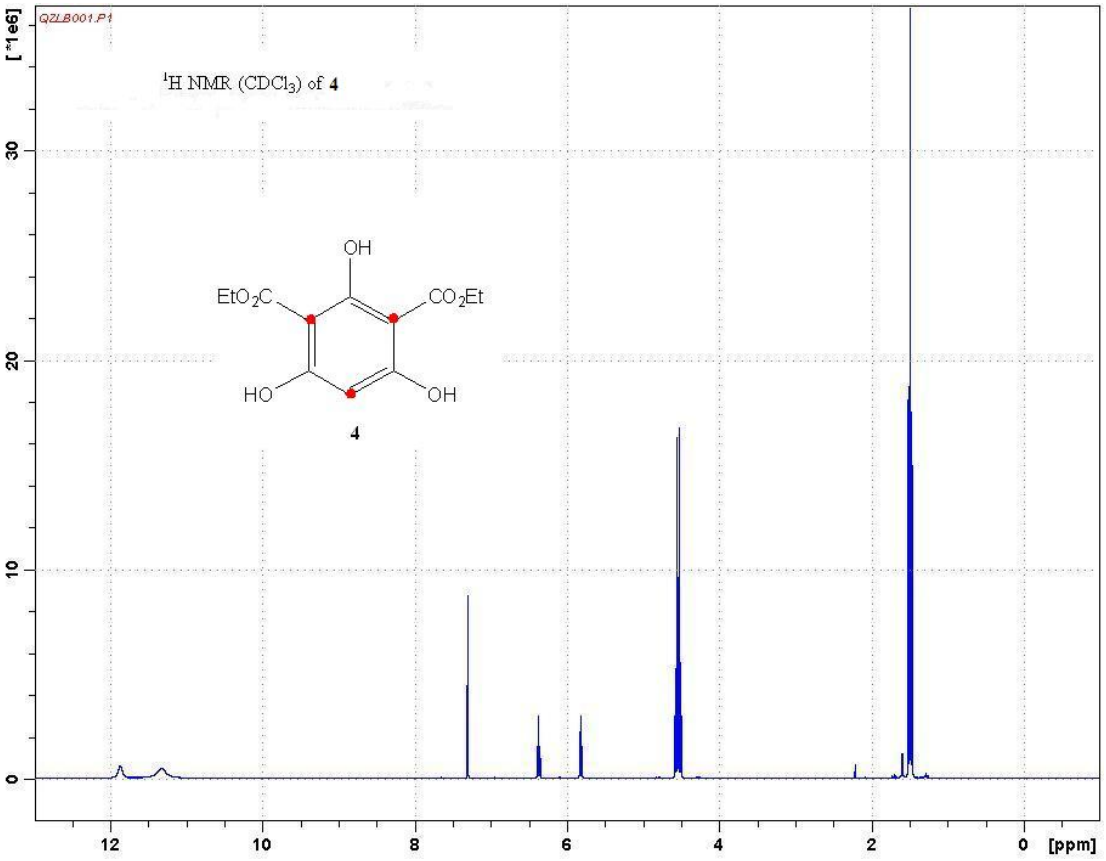
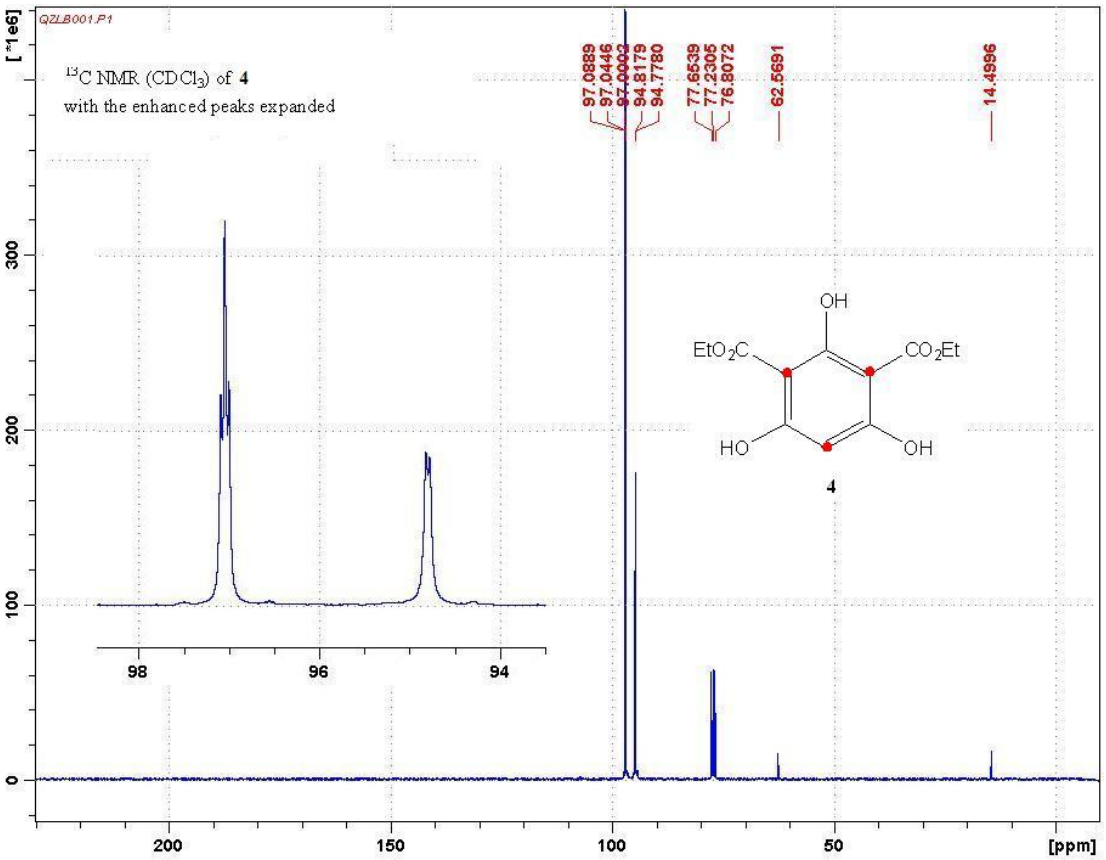
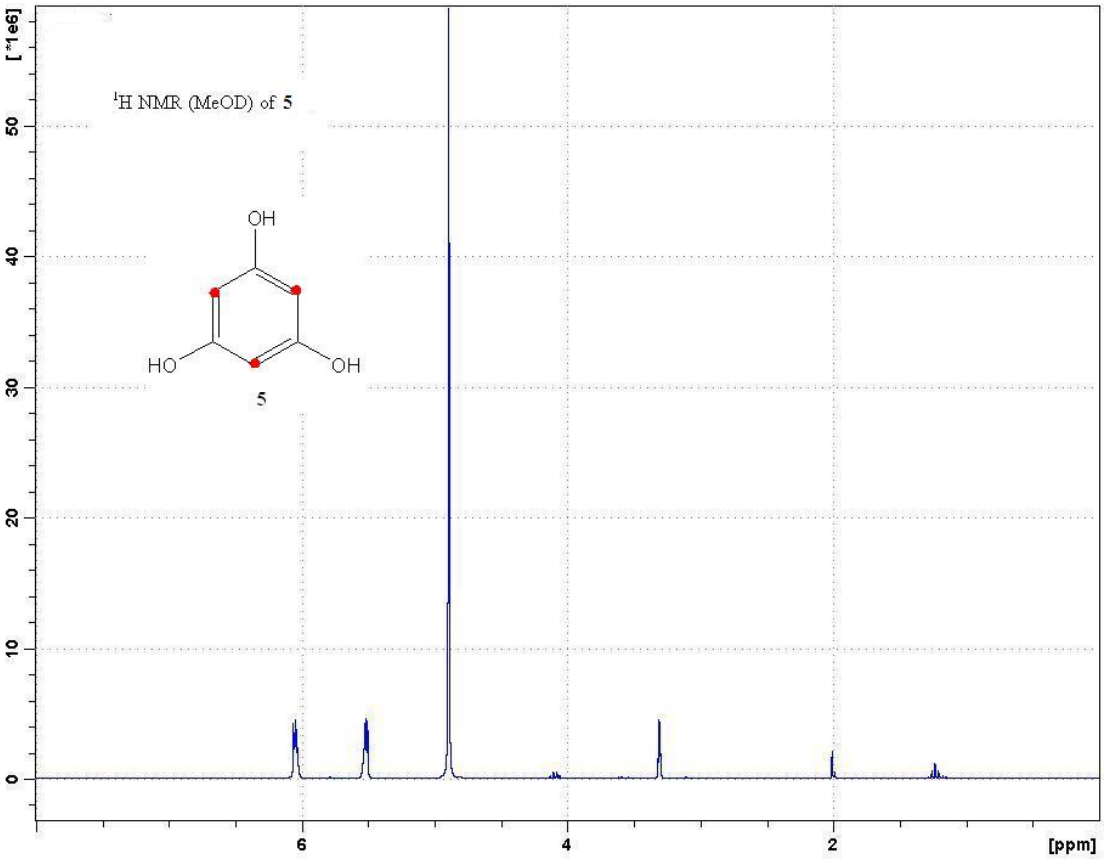
Selected ^{1}H-NMR and ^{13}C-NMR spectra



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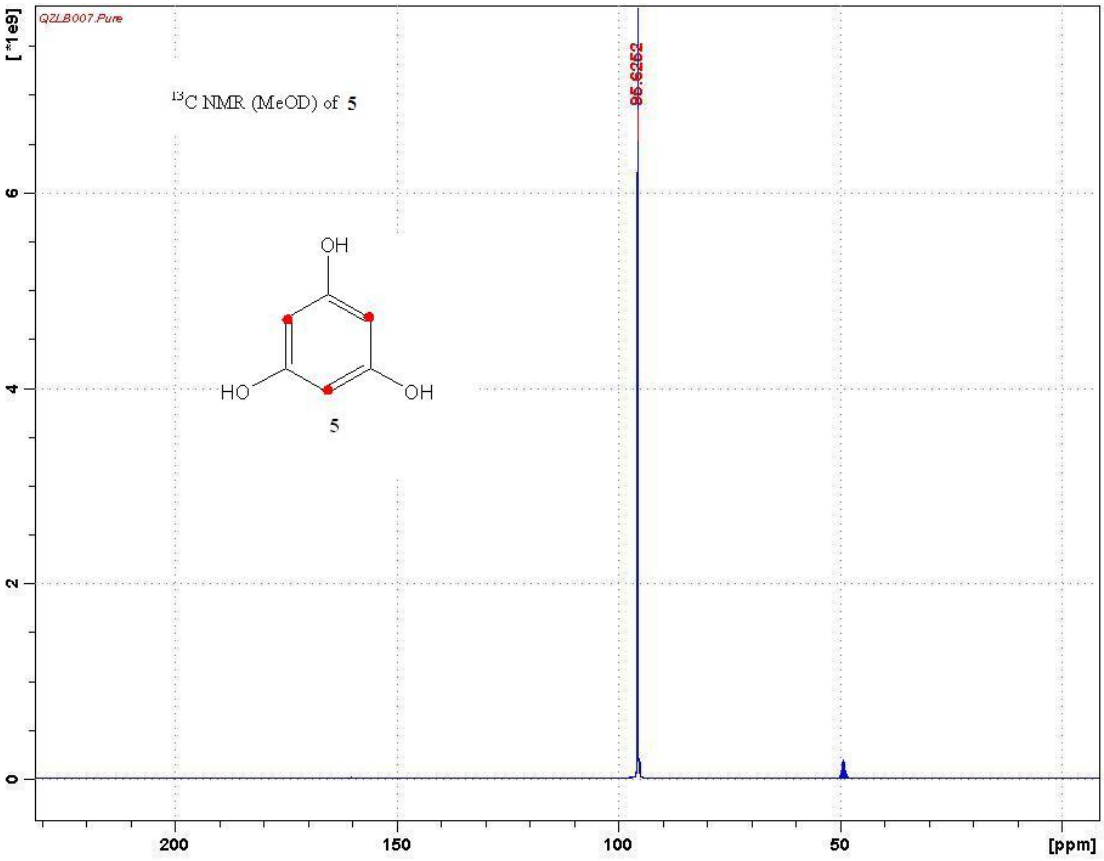
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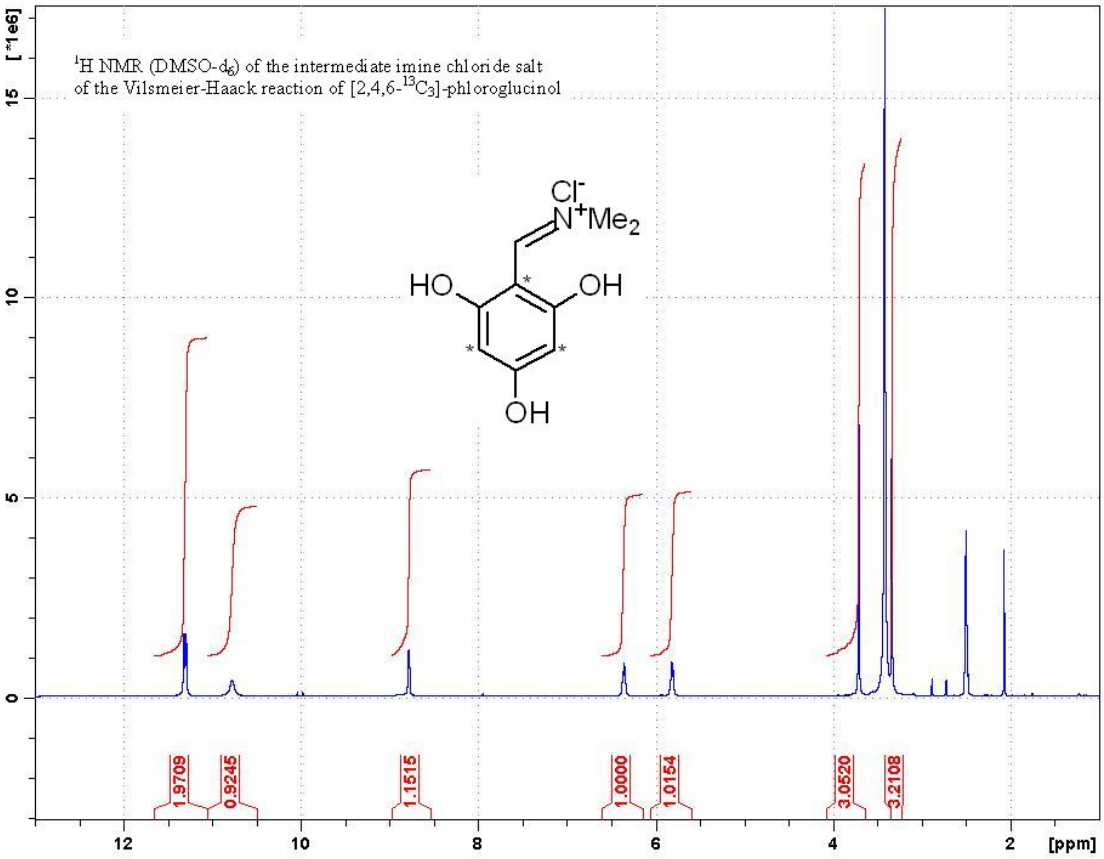
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N$^+$Me$_2$
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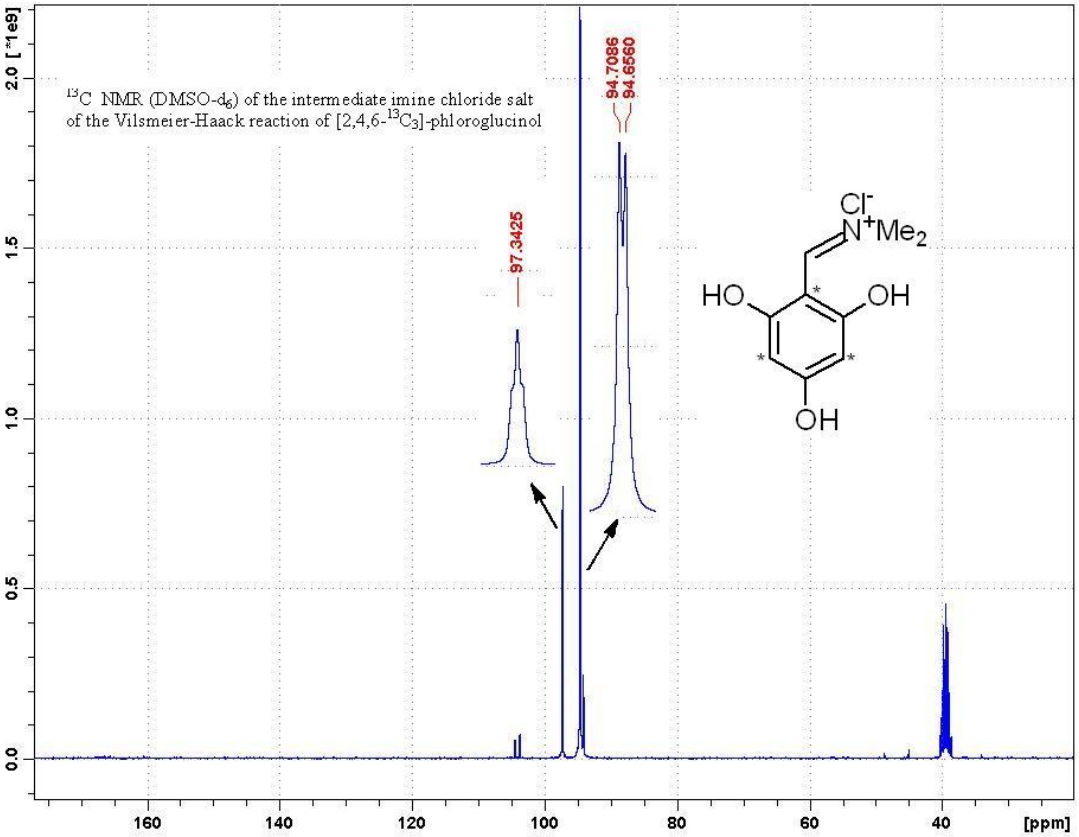
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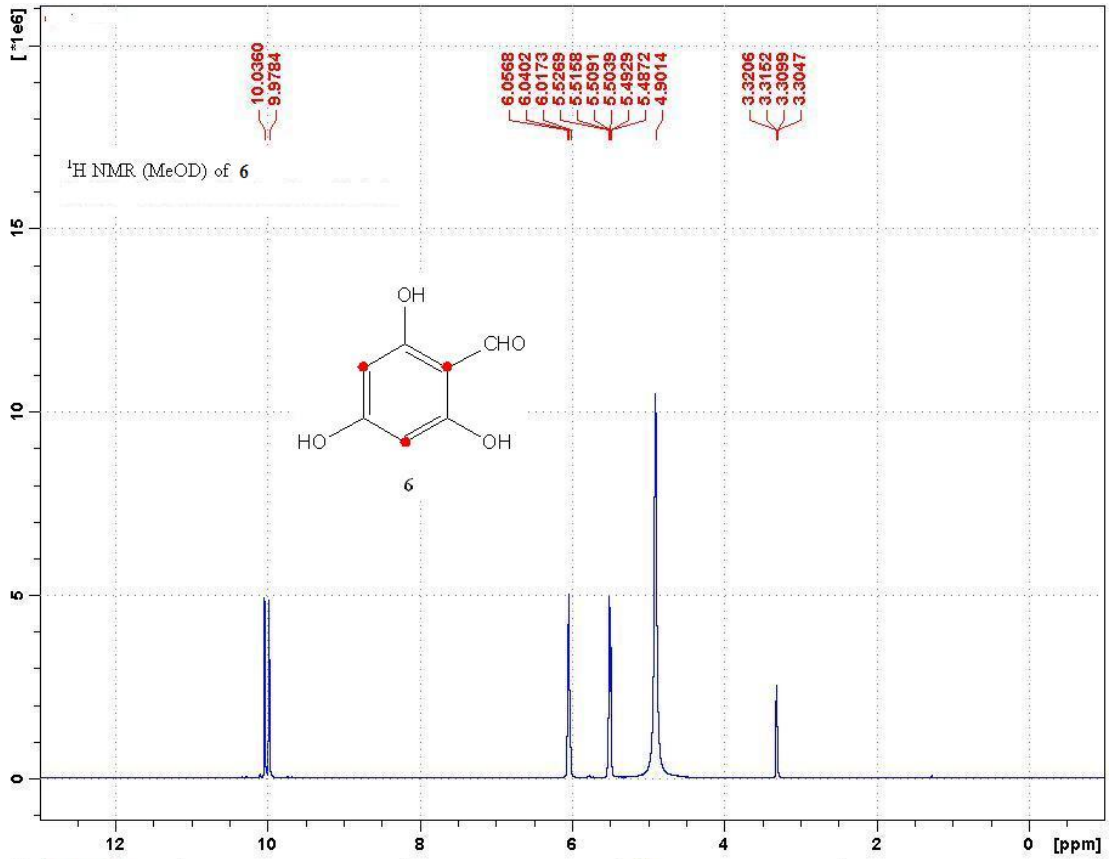
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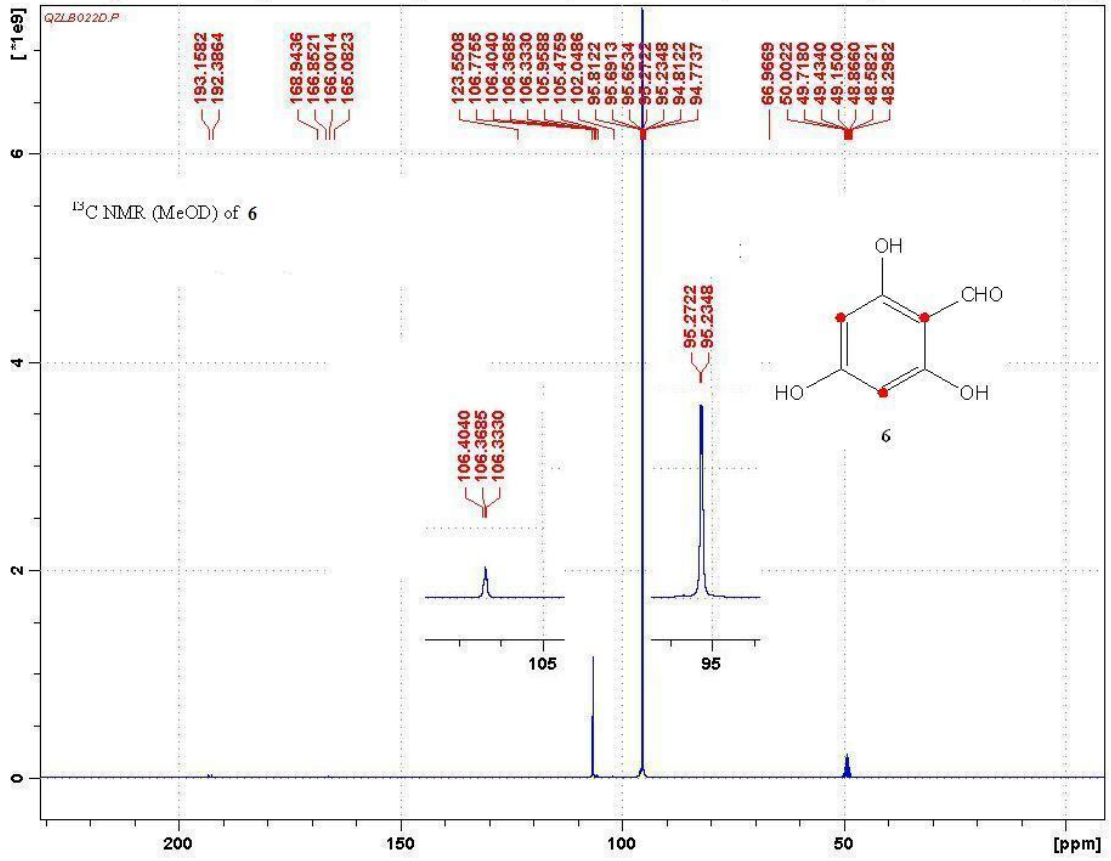
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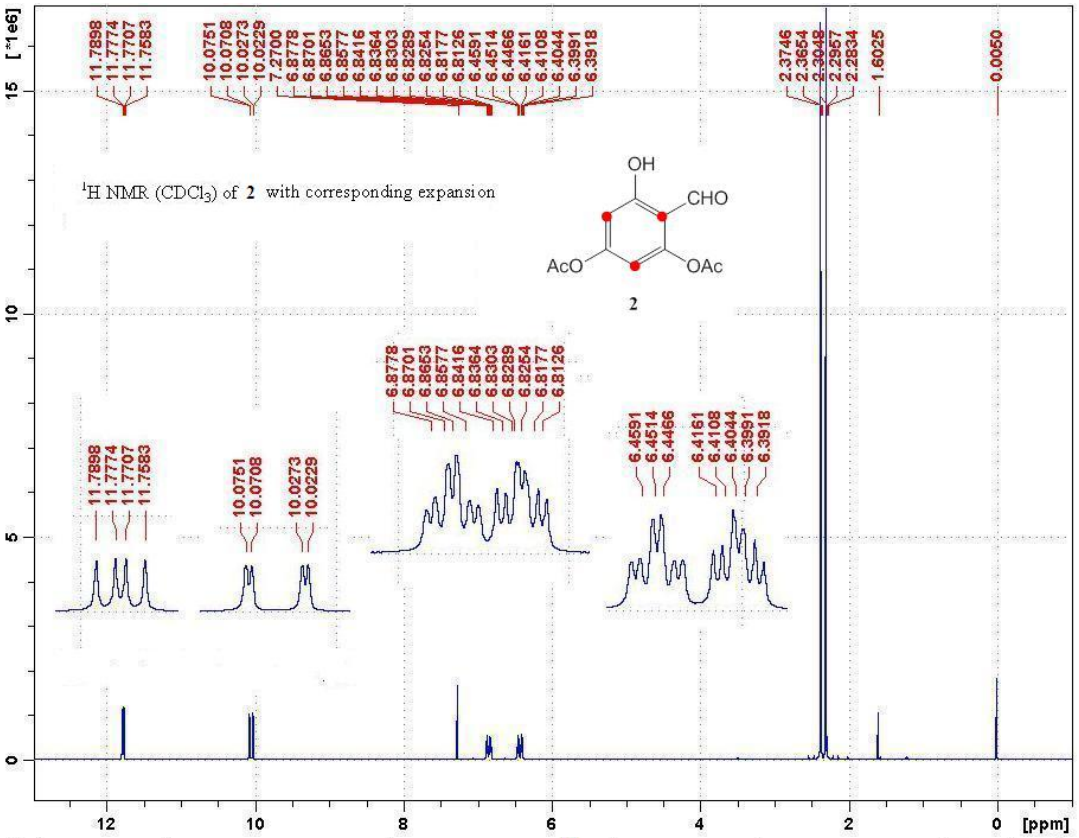
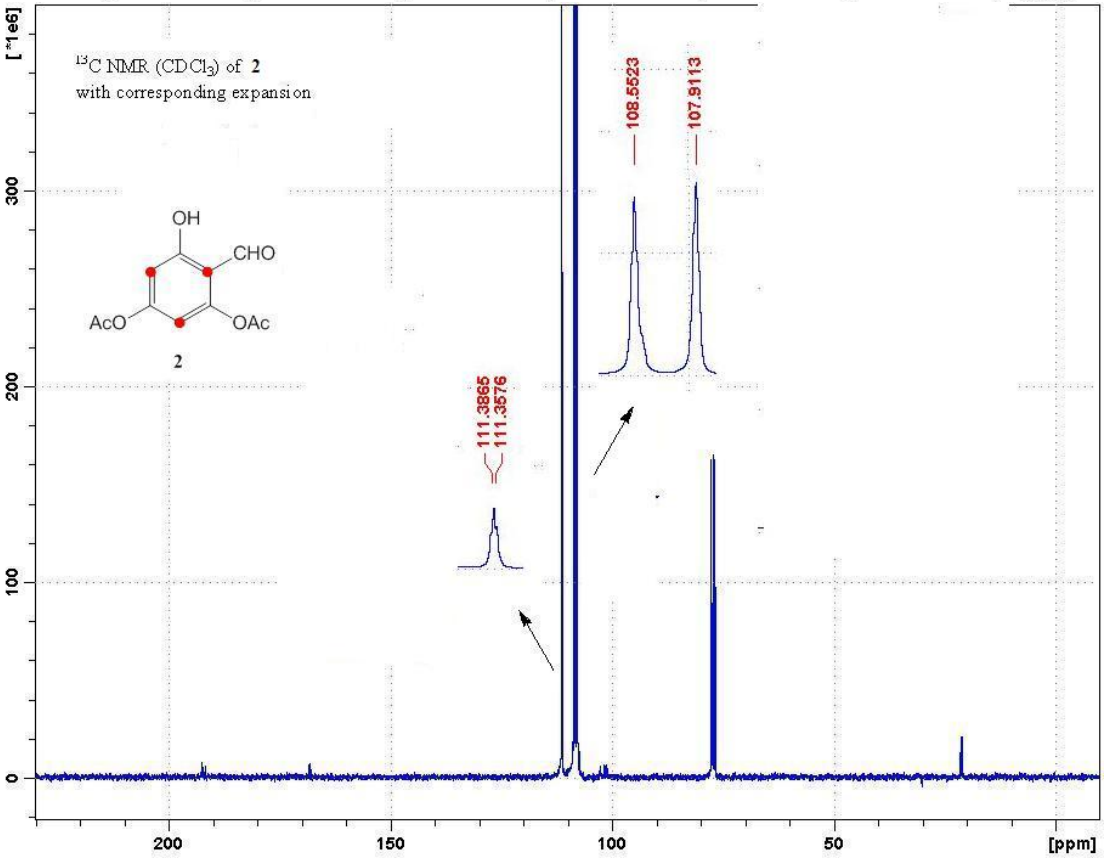
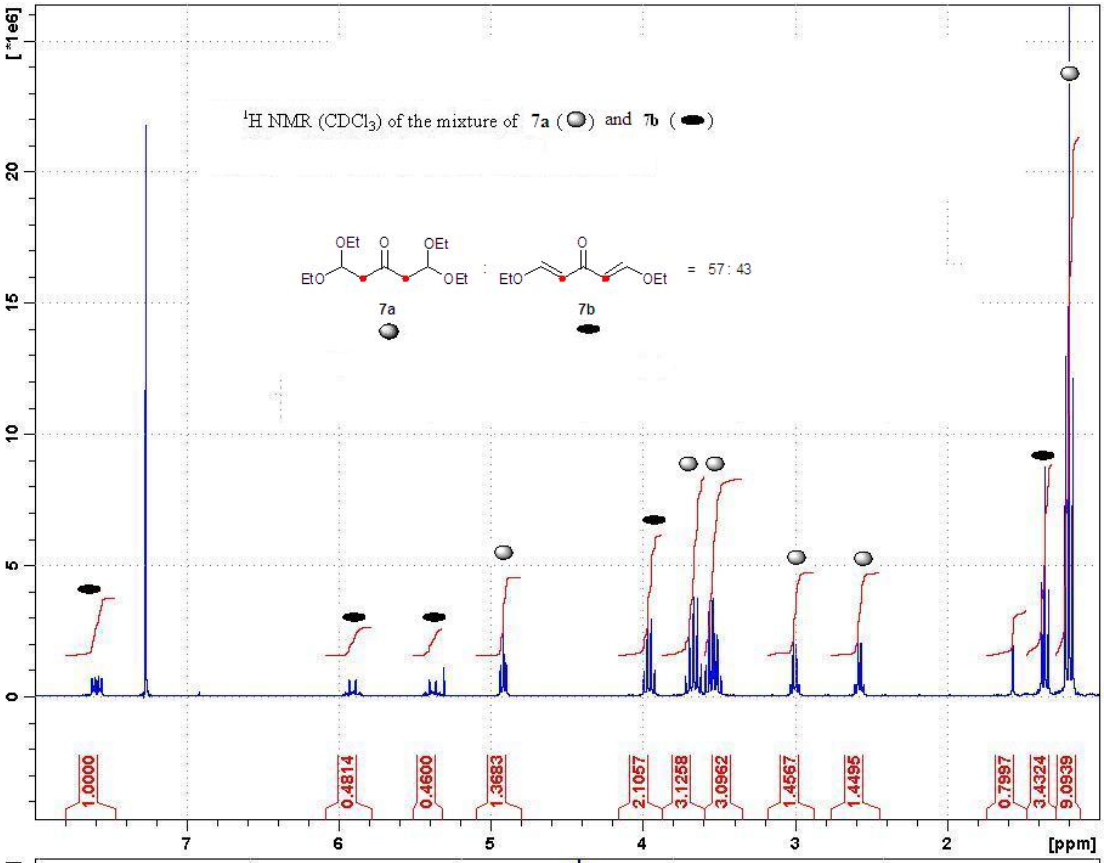
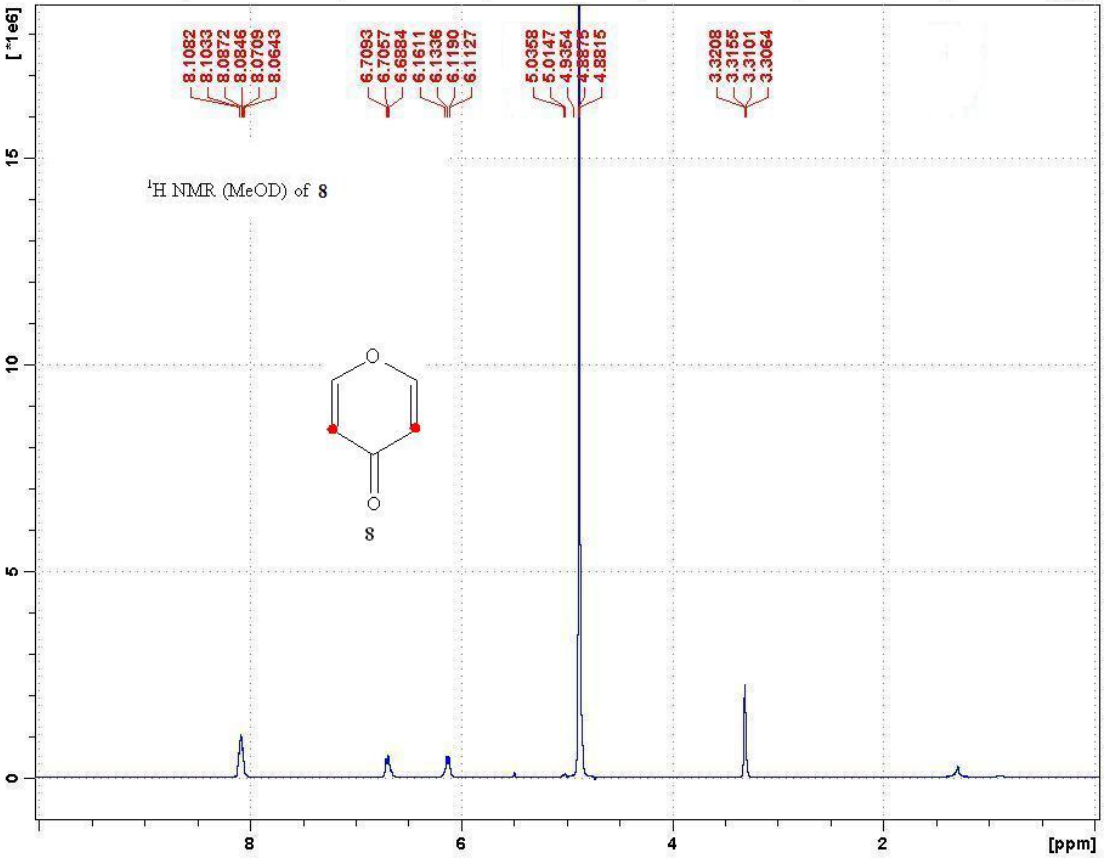
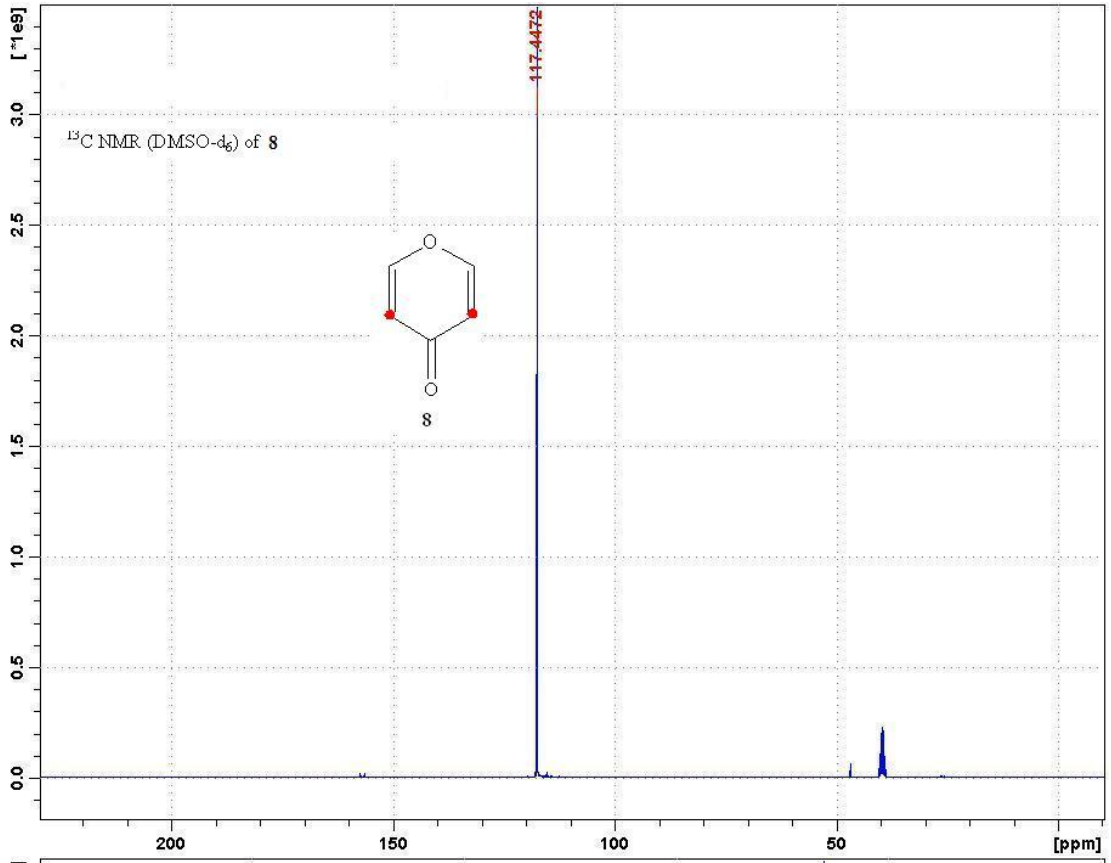
48.5821
48.2982
^{13}C NMR (MeOD) of 6
95.2722
95.2348
106.4040
106.3685
106.3330
OH
CHO
HO
OH
6
105
95
[ppm]

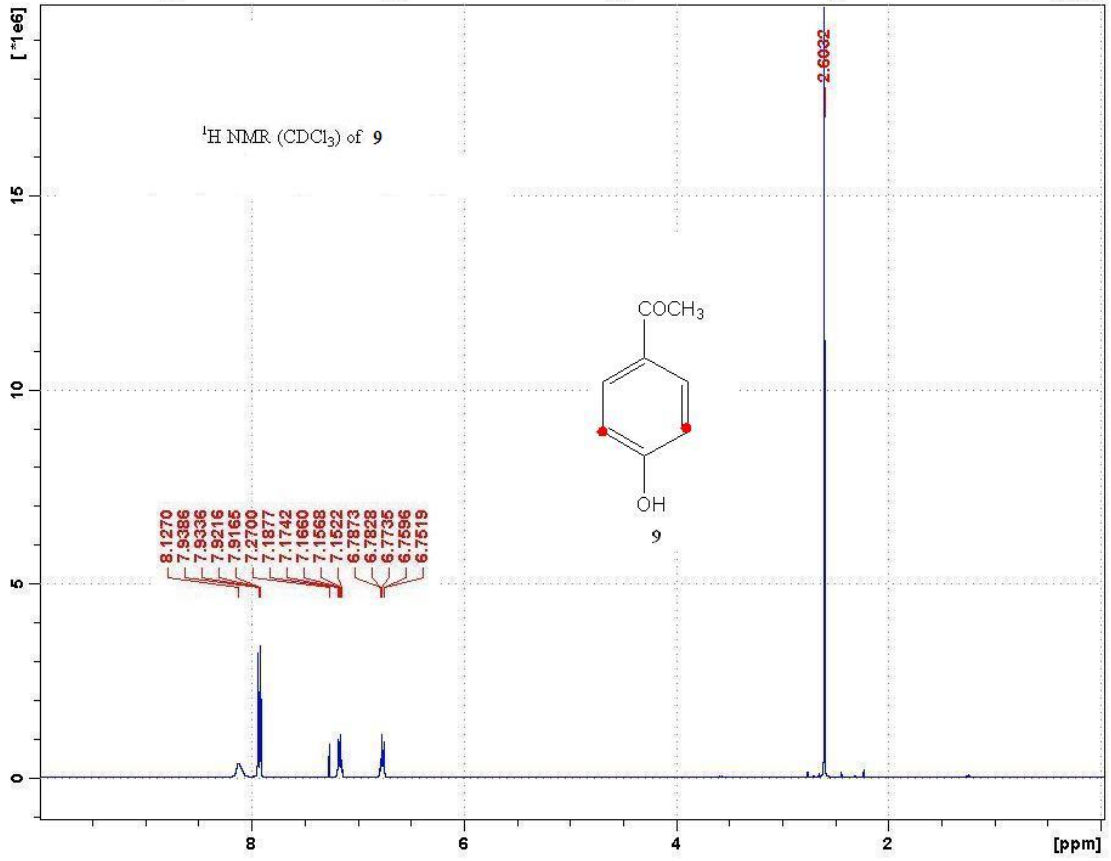

[*1e6]
15
10
5
0
11.7898
11.7774
11.7707
11.7583
10.0751
10.0708
10.0273
10.0229
7.2700
6.8778
6.8701
6.8653
6.8577
6.8416
6.8364
6.8303
6.8289
6.8254
6.8177
6.8126
6.4591
6.4514
6.4466
6.4161
6.4108
6.4044
6.3991
6.3918
2.3746
2.3654
2.3048
2.2957
2.2834
1.6025
0.0050
^{1}H NMR (CDCl$_3$) of 2 with corresponding expansion
OH
CHO
AcO
OAc
2
12
10
8
6
4
2
0
[ppm]

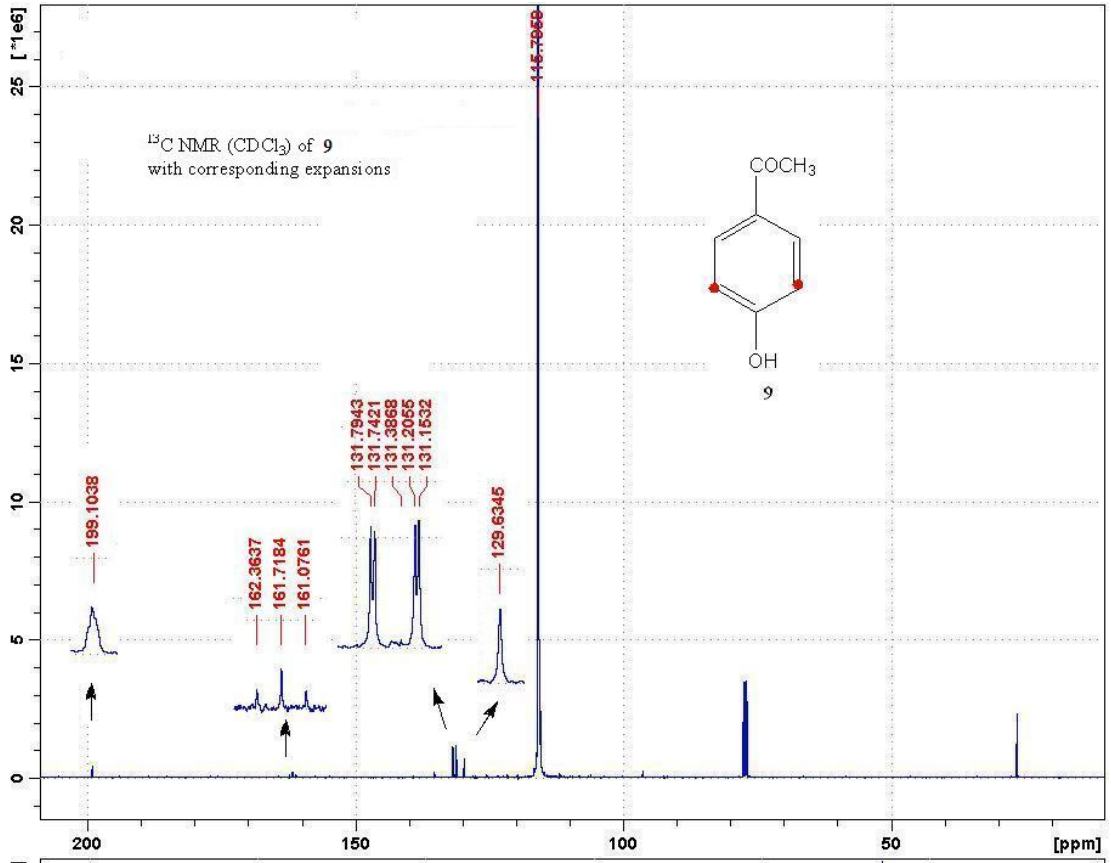

[*1e6]
300
200
100
0
^{13}C NMR (CDCl$_3$) of 2
with corresponding expansion
108.5523
107.9113
111.3865
111.3576
OH
CHO
AcO
OAc
2
200
150
100
50
[ppm]

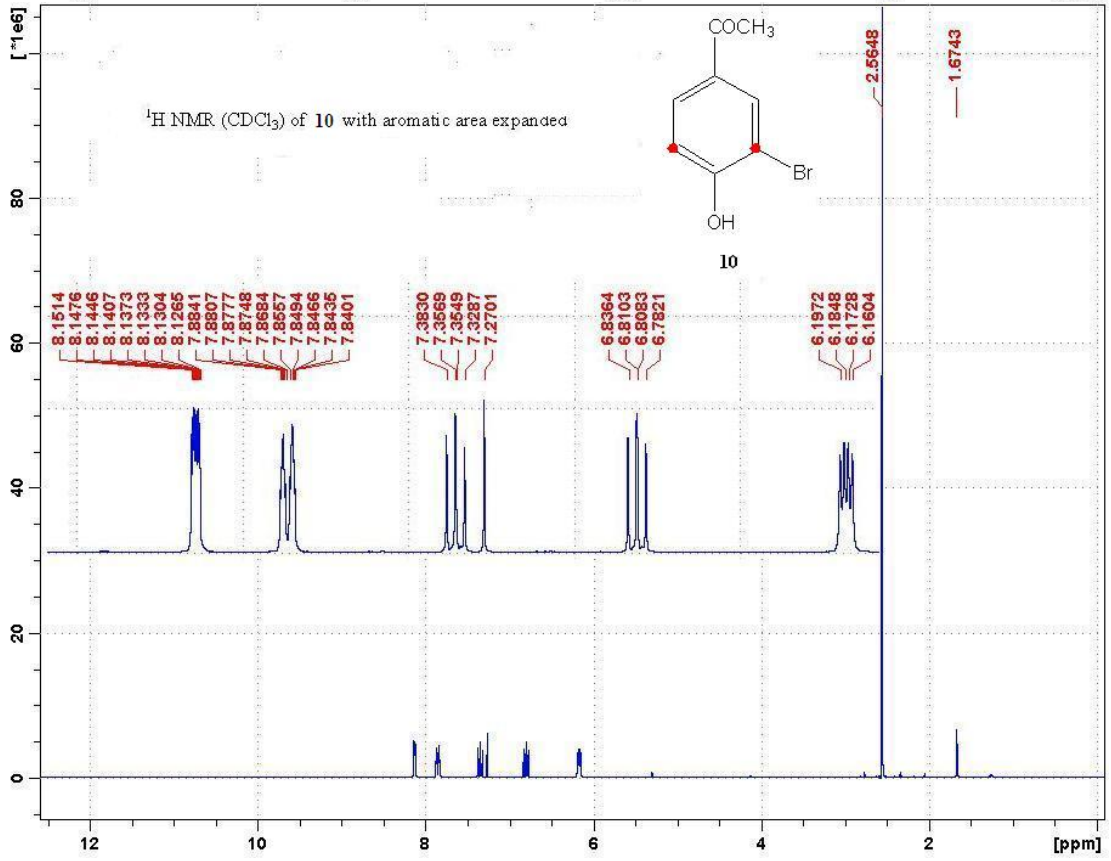

[*1e6]
^{1}H NMR ($CDCl_3$) of the mixture of 7a ( ) and 7b ( )
OEt
O
OEt
EtO
OEt
7a
7b
= 57 : 43
1.0000
0.4814
0.4600
1.3683
2.1057
3.1258
3.0962
1.4567
1.4495
0.7997
3.4324
9.0939
[ppm]

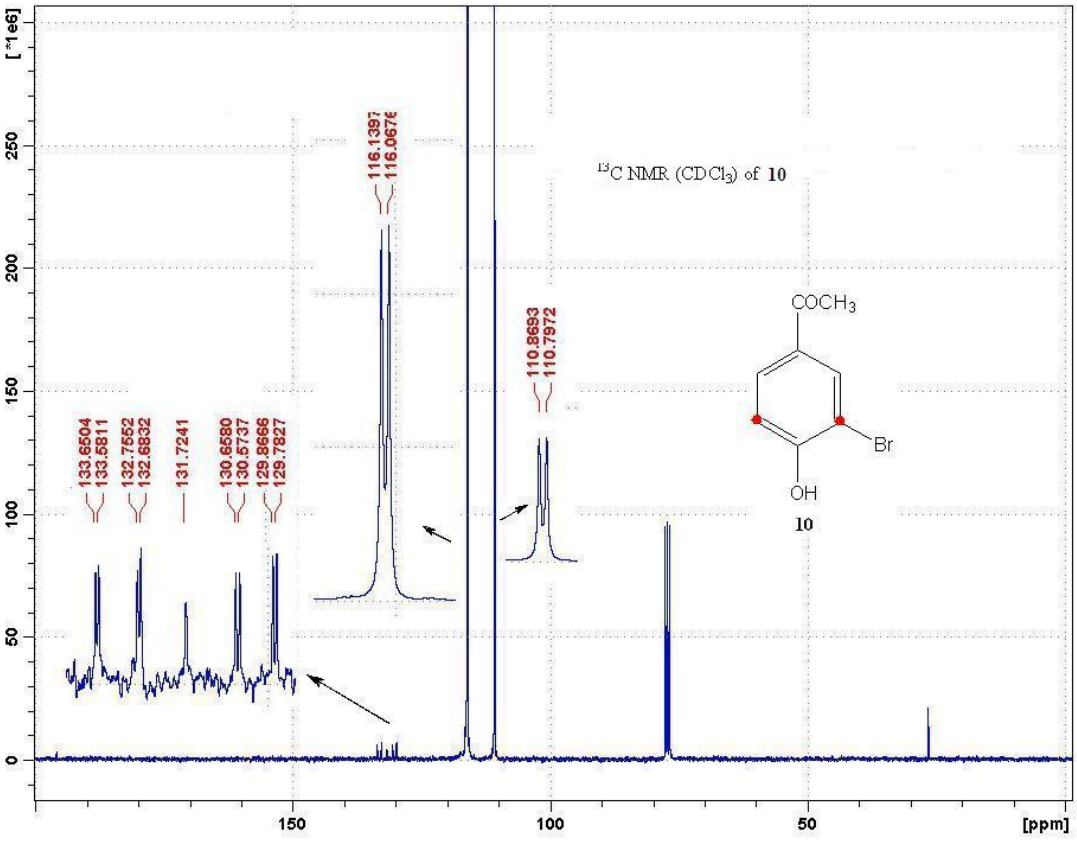

[*1e6]
8.1082
8.1033
8.0872
8.0846
8.0709
8.0643
6.7093
6.7057
6.6884
6.1611
6.1336
6.1190
6.1127
5.0358
5.0147
4.9354
4.8875
4.8815
3.3208
3.3155
3.3101
3.3064
^{1}H NMR (MeOD) of 8
O
O
8
[ppm]

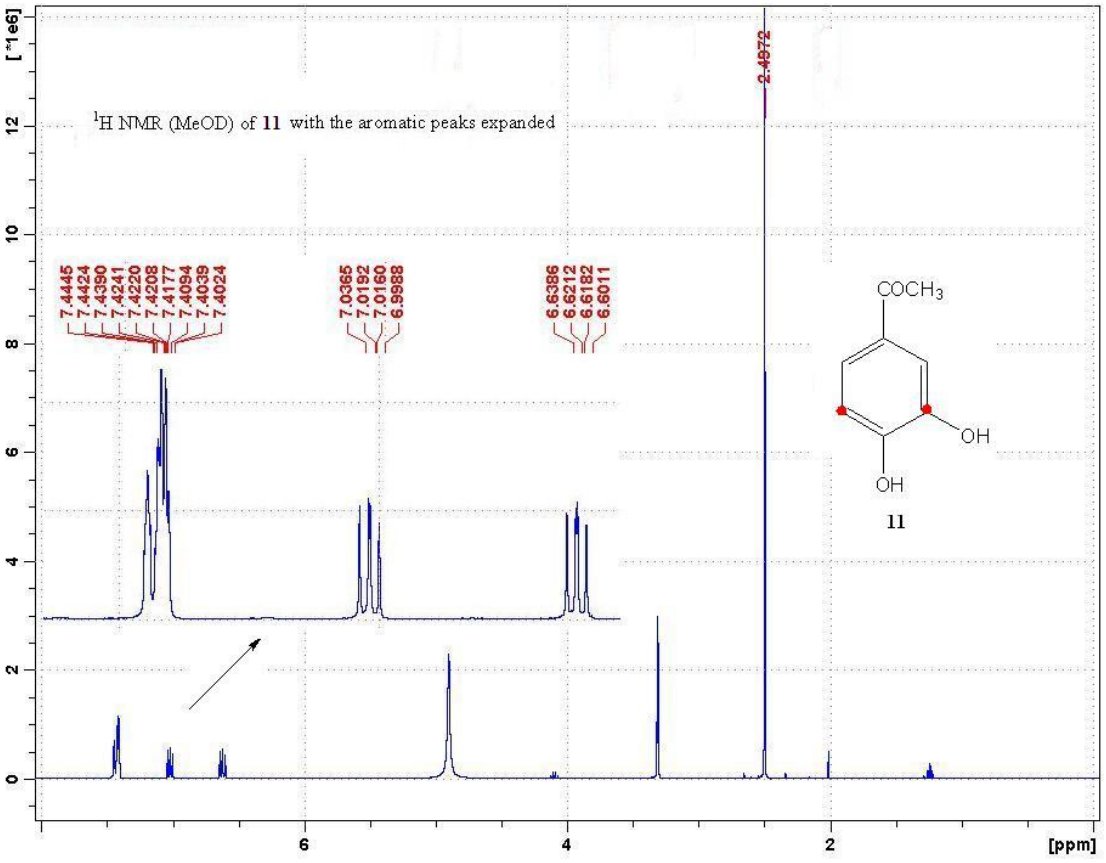

[*1e9]
3.0
2.5
2.0
1.5
1.0
0.5
0.0
117.4472
^{13}C NMR (DMSO-d_6) of 8
O
O
8
200
150
100
50
[ppm]

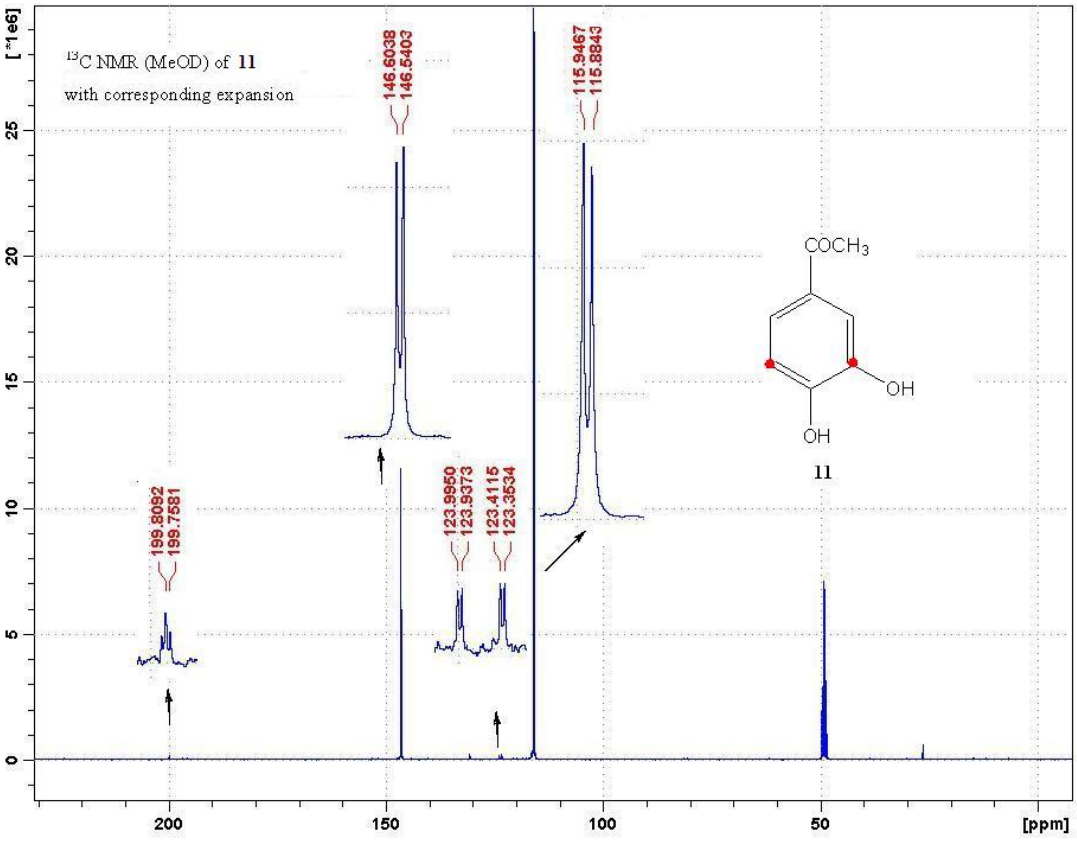

[*1e6]
15
10
5
0
2.6032
^{1}H NMR ($CDCl_3$) of 9
$COCH_3$
OH
9
8.1270
7.9386
7.9336
7.9216
7.9165
7.2700
7.1877
7.1742
7.1660
7.1568
7.1522
6.7873
6.7828
6.7735
6.7596
6.7519
8
6
4
2
[ppm]

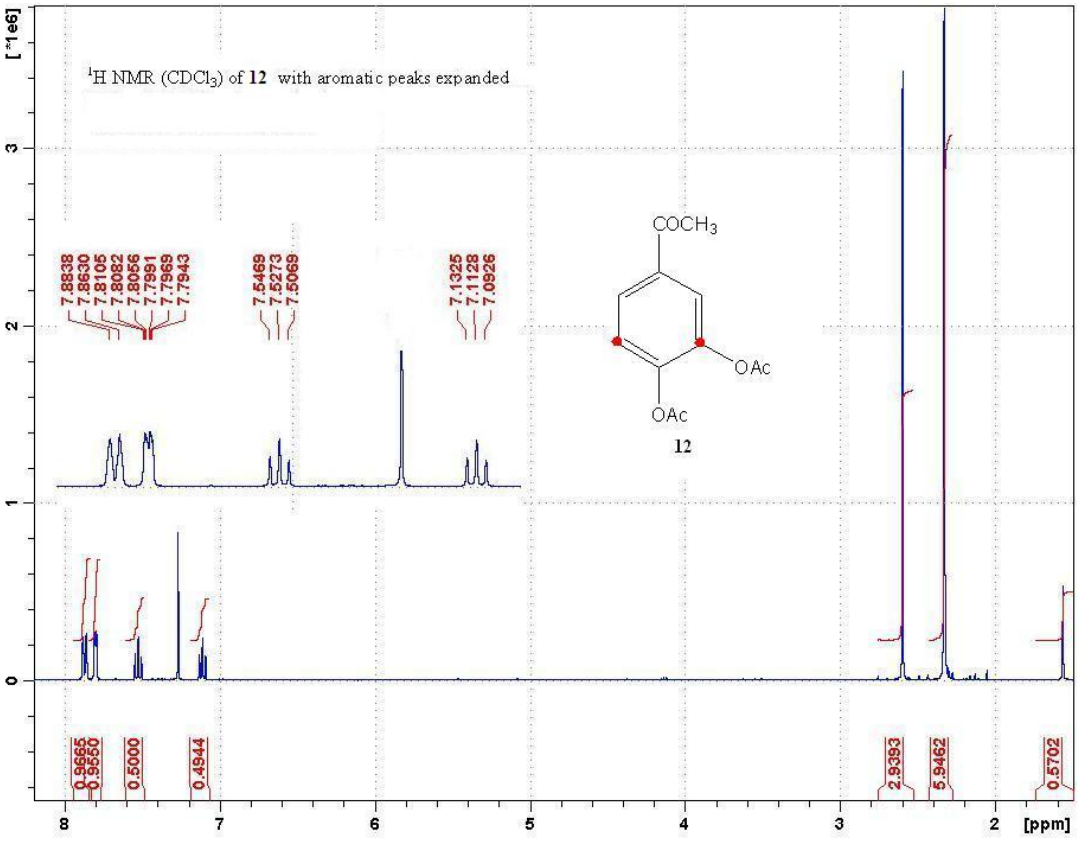

^{13}C NMR ($CDCl_3$) of 9
with corresponding expansions
COCH3
OH
9
199.1038
162.3637
161.7184
161.0761
131.7943
131.7421
131.3868
131.2055
131.1532
129.6345
116.7868
[*1e6]
[ppm]

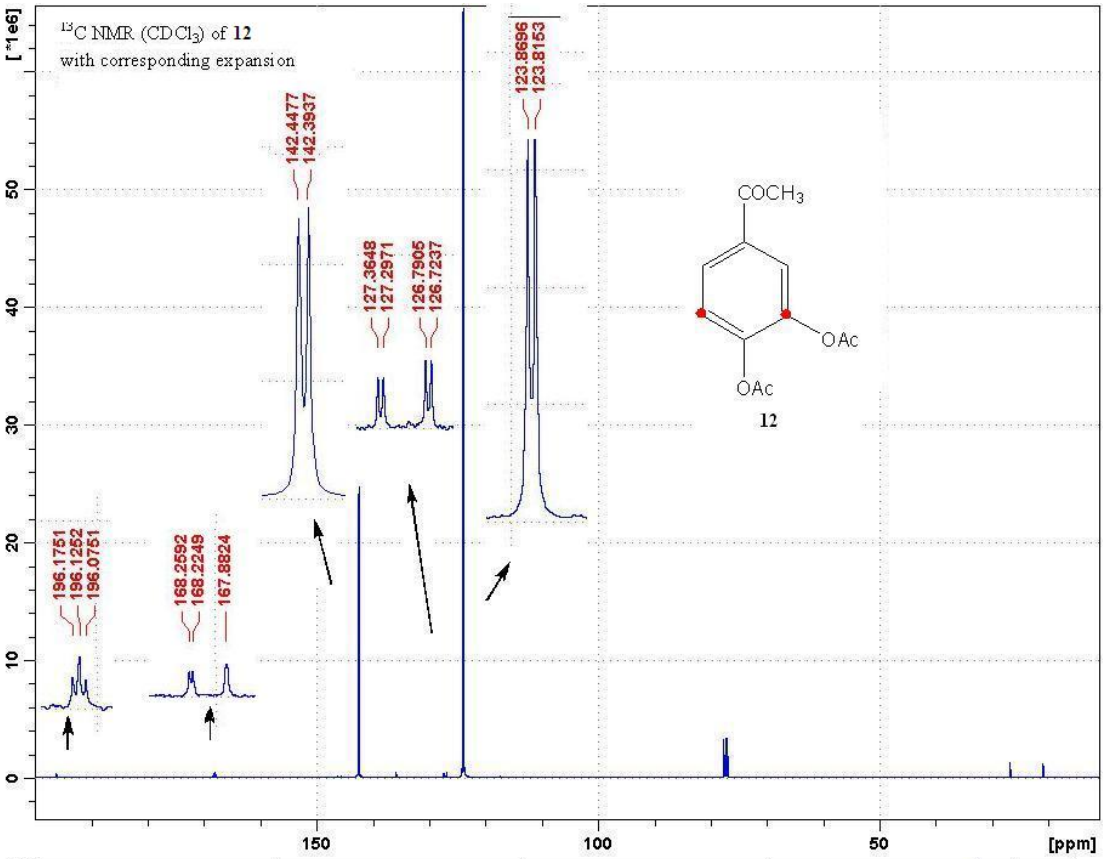

^{1}H NMR ($CDCl_3$) of 10 with aromatic area expanded
COCH3
Br
OH
10
2.5648
1.6743
8.1514
8.1476
8.1446
8.1407
8.1373
8.1333
8.1304
8.1265
7.8841
7.8807
7.8777
7.8748
7.8684
7.8557
7.8494
7.8466
7.8435
7.8401
7.3830
7.3569
7.3549
7.3287
7.2701
6.8364
6.8103
6.8083
6.7821
6.1972
6.1848
6.1728
6.1604
[*1e6]
[ppm]

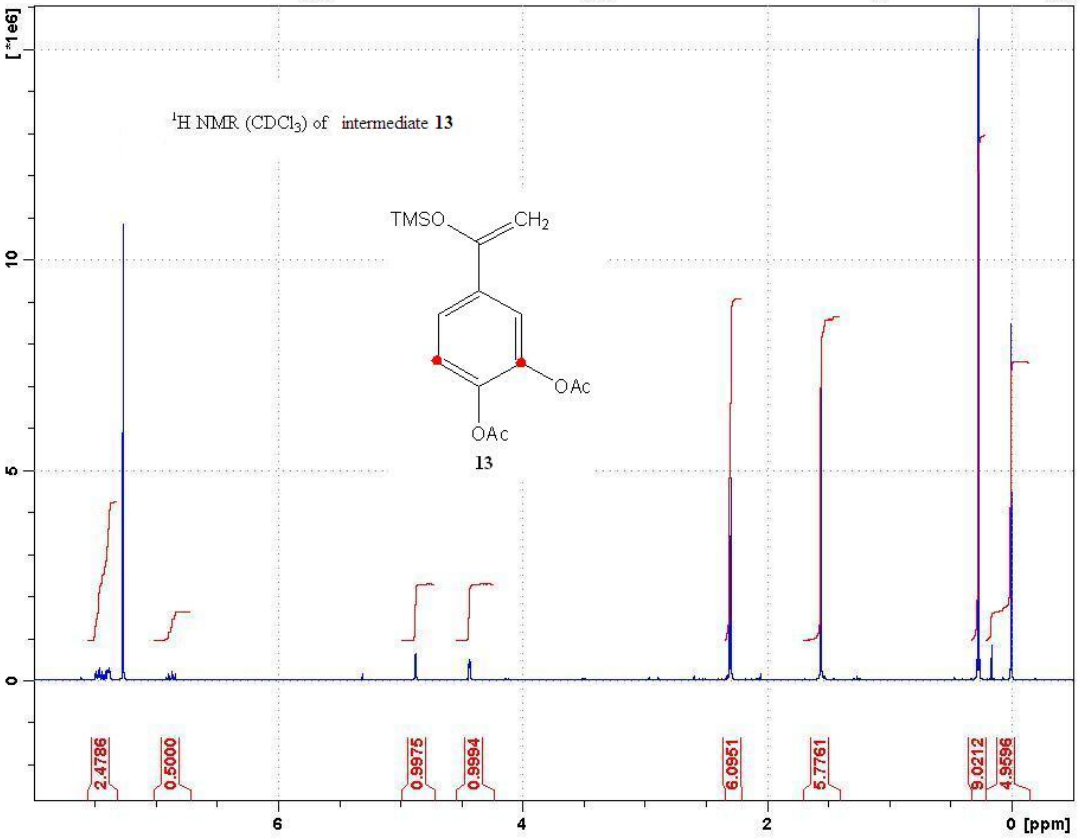

[*1e6]
250
200
150
100
50
0
116.1397
116.0678
110.8693
110.7972
133.6504
133.5811
132.7552
132.6832
131.7241
130.6580
130.5737
129.8866
129.7827
150
100
50
[ppm]
^{13}C NMR (CDCl$_3$) of 10
COCH$_3$
Br
OH
10

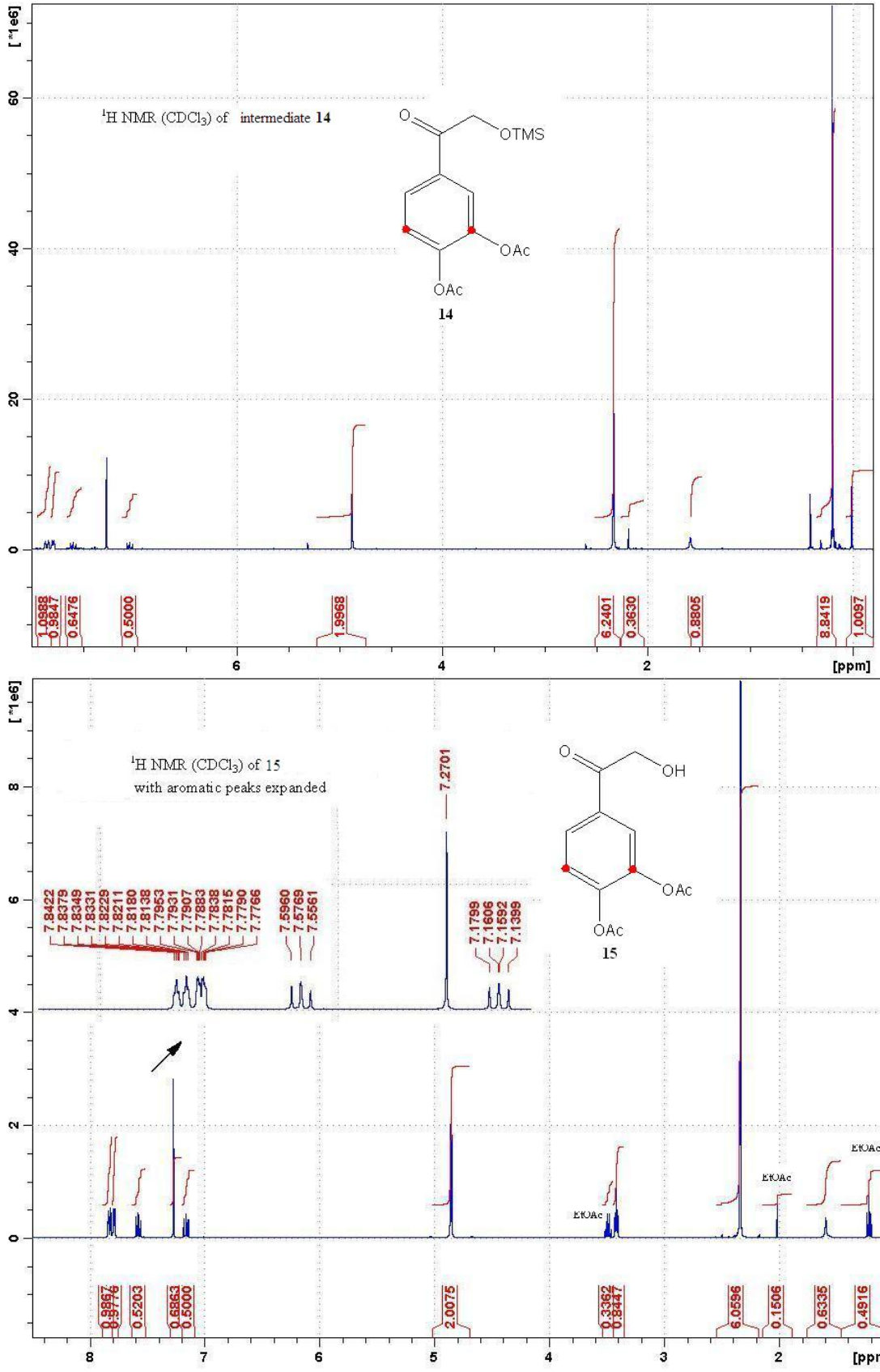

[*1e6]
12
10
8
6
4
2
0
2.4972
7.4445
7.4424
7.4390
7.4241
7.4220
7.4208
7.4177
7.4094
7.4039
7.4024
7.0365
7.0192
7.0160
6.9988
6.6386
6.6212
6.6182
6.6011
6
4
2
[ppm]
^{1}H NMR (MeOD) of 11 with the aromatic peaks expanded
COCH$_3$
OH
OH
11

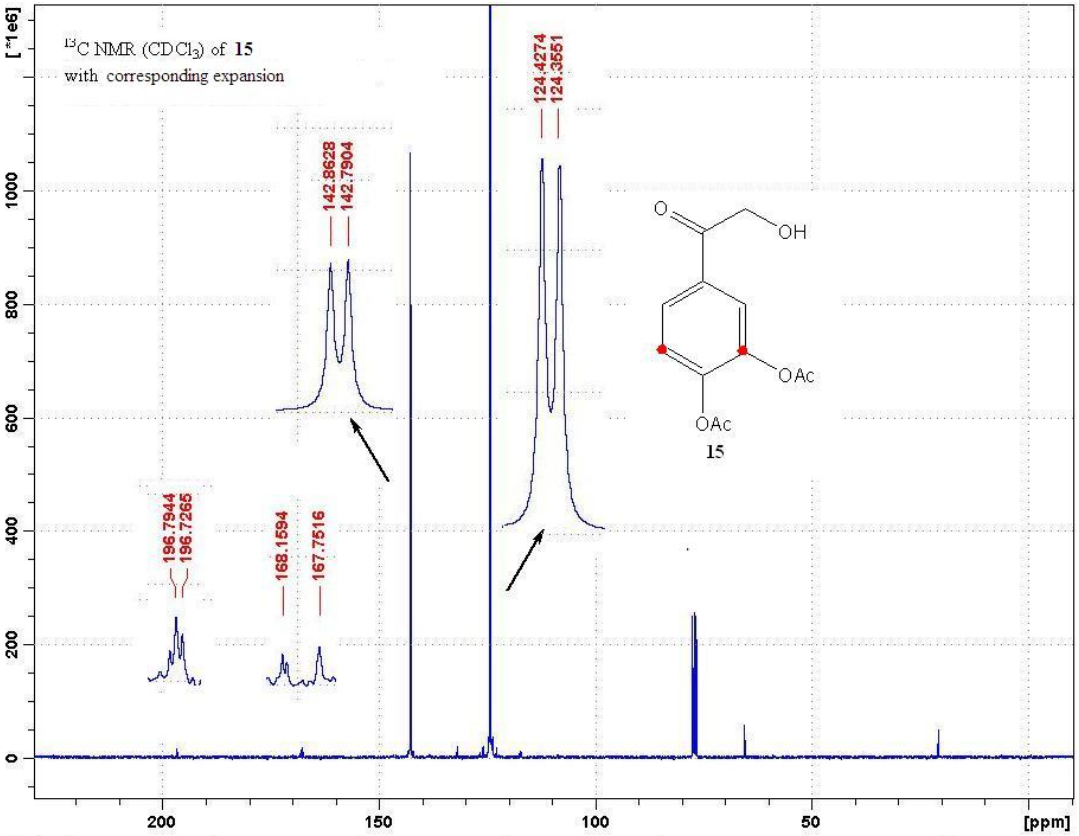

^{13}C NMR (MeOD) of 11
with corresponding expansion
146.6038
146.5403
115.9467
115.8843
199.8092
199.7581
123.9950
123.8373
123.4115
123.3534
COCH3
OH
OH
11
[*1e6]
[ppm]

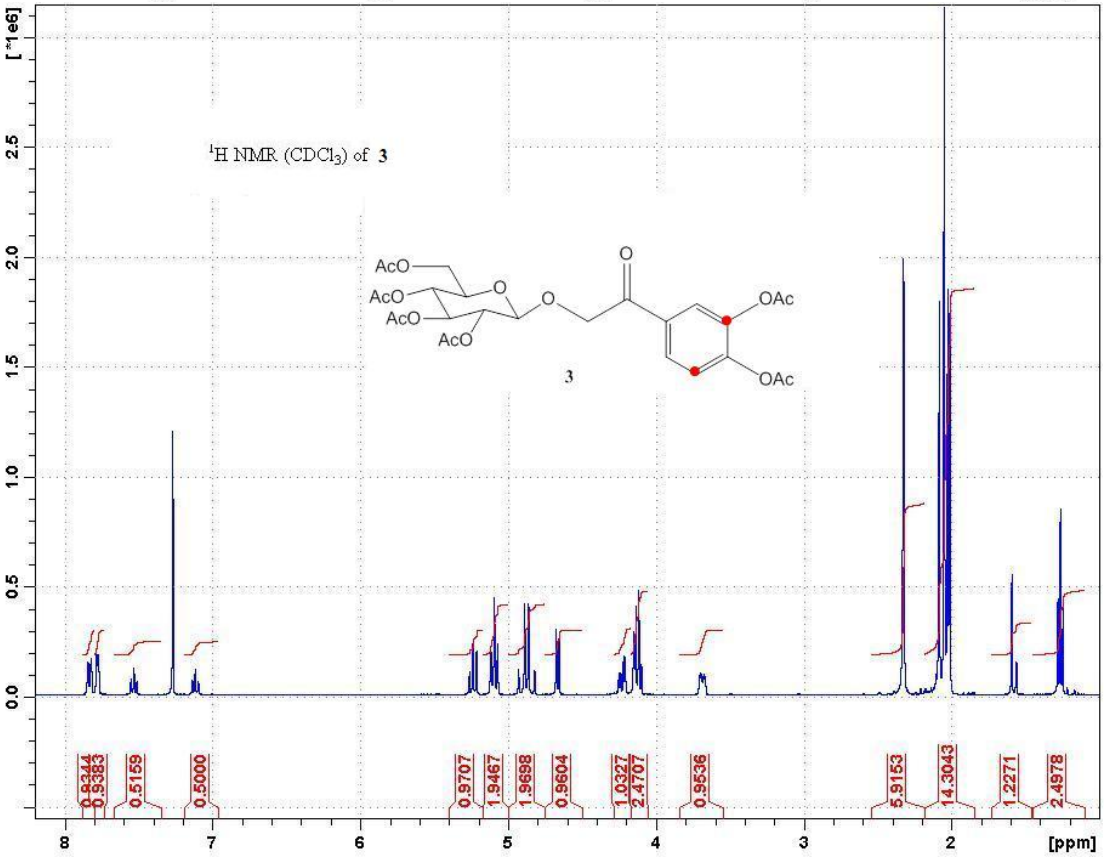

^{1}H NMR (CDCl$_3$) of 12 with aromatic peaks expanded
7.8838
7.8630
7.8105
7.8082
7.8056
7.7991
7.7969
7.7943
7.5469
7.5273
7.5069
7.1325
7.1128
7.0926
COCH3
OAc
OAc
12
0.9665
0.9550
0.5000
0.4944
2.9393
5.9462
0.5702
[*1e6]
[ppm]

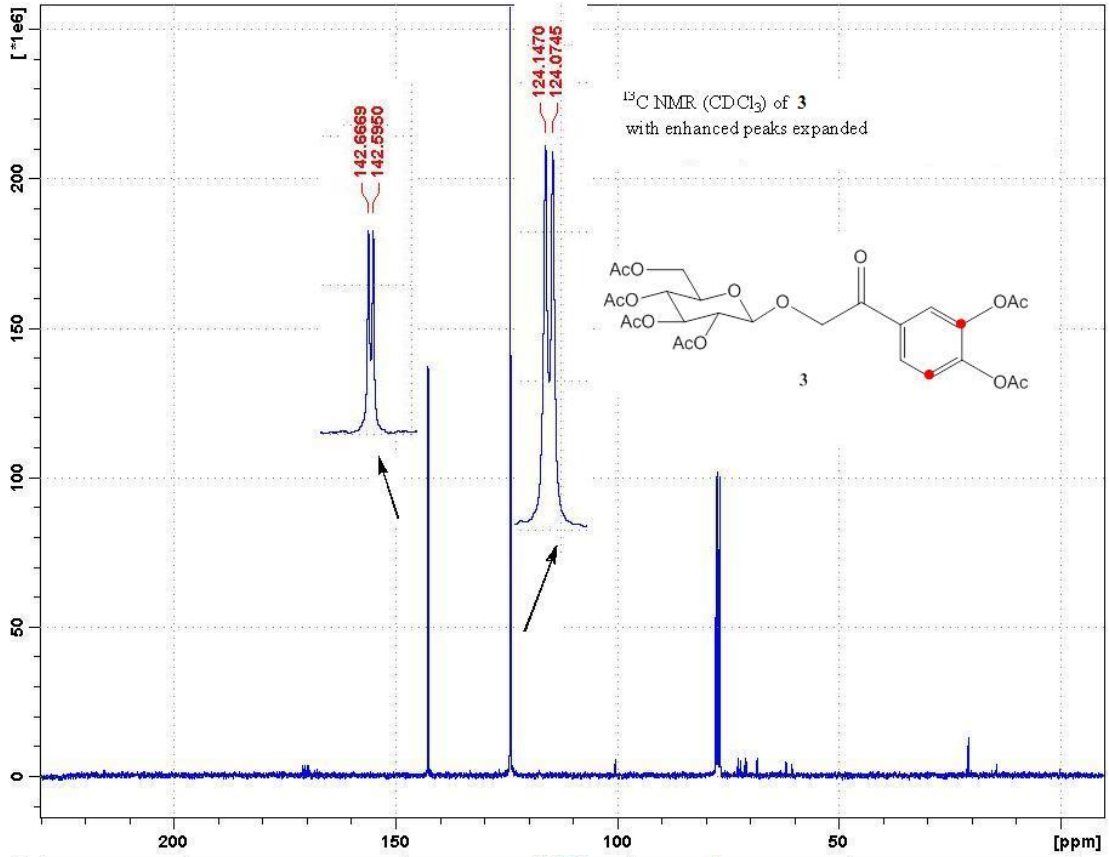

^{13}C NMR ($CDCl_3$) of 12
with corresponding expansion
[*1e6]
142.4477
142.3937
127.3648
127.2971
126.7905
126.7237
123.8696
123.8153
196.1751
196.1252
196.0751
168.2592
168.2249
167.8824
COCH3
OAc
OAc
12
150
100
50
[ppm]

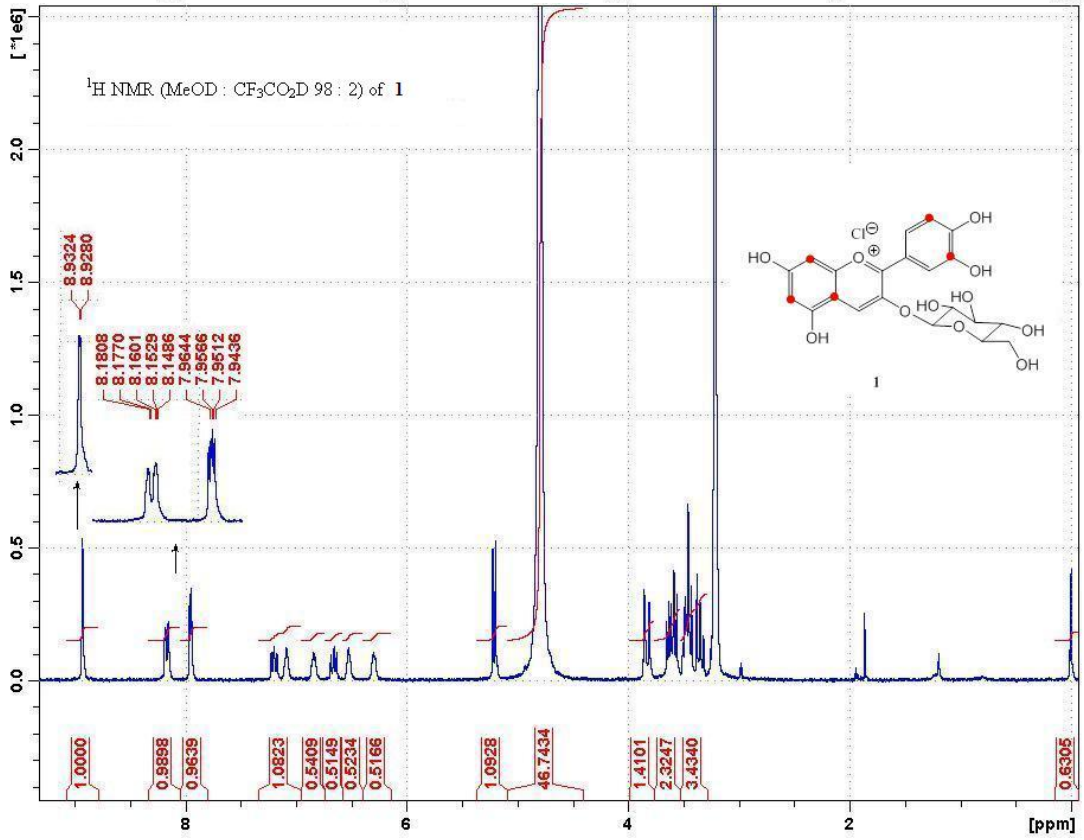

^{1}H NMR ($CDCl_3$) of intermediate 13
[*1e6]
TMSO
CH2
OAc
OAc
13
2.4786
0.5000
0.9975
0.9994
6.0951
5.7761
9.0212
4.9596
6
4
2
0 [ppm]

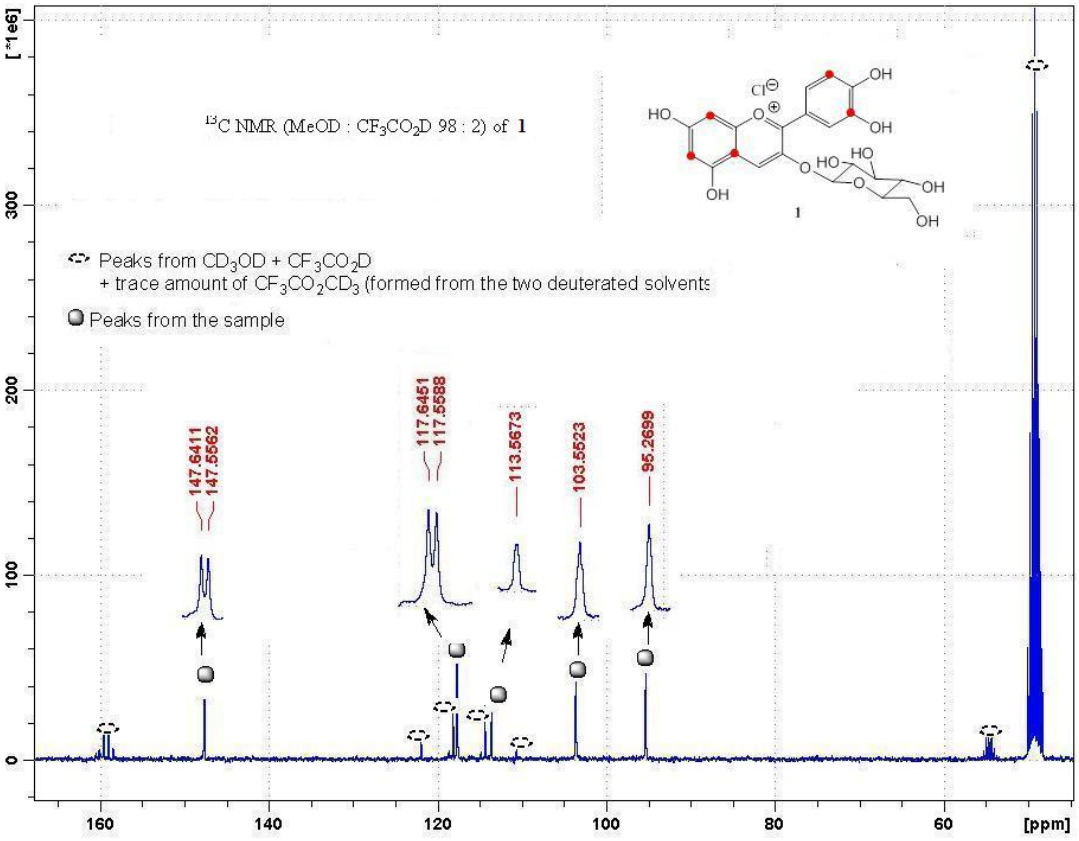

[*1e6]
^{1}H NMR ($CDCl_3$) of intermediate 14
O
OTMS
OAc
OAc
14
1.0988
0.9847
0.6476
0.5000
1.9968
6.2401
0.3630
0.8805
8.8419
1.0097
[ppm]
[*1e6]
^{1}H NMR ($CDCl_3$) of 15
with aromatic peaks expanded
7.2701
7.8422
7.8379
7.8348
7.8331
7.8229
7.8211
7.8180
7.8138
7.7953
7.7931
7.7907
7.7883
7.7838
7.7815
7.7790
7.7766
7.5960
7.5769
7.5561
7.1799
7.1606
7.1592
7.1399
O
OH
OAc
OAc
15
EtOAc
EtOAc
EtOAc
0.9887
0.9776
0.5203
0.6863
0.5000
2.0075
0.3362
0.8447
6.0596
0.1506
0.6335
0.4916
[ppm]


^{13}C NMR ($CDCl_3$) of 15
with corresponding expansion
142.8628
142.7904
124.4274
124.3551
196.7944
196.7265
168.1594
167.7516
OH
OAc
OAc
15
[*1e6]
[ppm]


^{1}H NMR ($CDCl_3$) of 3
AcO
AcO
AcO
AcO
OAc
OAc
3
0.9344
0.9383
0.5159
0.5000
0.9707
1.9467
1.9698
0.9604
1.0327
2.4707
0.9536
5.9153
14.3043
1.2271
2.4978
[*1e6]
[ppm]


[*1e6]
200
150
100
50
0
142.6669
142.5950
124.1470
124.0745
^{13}C NMR (CDCl$_3$) of 3
with enhanced peaks expanded
AcO
AcO
AcO
AcO
O
O
O
OAc
OAc
3
[ppm]


[*1e6]
2.0
1.5
1.0
0.5
0.0
^{1}H NMR (MeOD : CF$_3$CO$_2$D 98 : 2) of 1
8.9324
8.9280
8.1808
8.1770
8.1601
8.1529
8.1486
7.9644
7.9566
7.9512
7.9436
Cl$^{\ominus}$
HO
OH
OH
OH
HO
HO
OH
OH
1
1.0000
0.9898
0.9639
1.0823
0.5409
0.5149
0.5234
0.5166
1.0928
46.7434
1.4101
2.3247
3.4340
0.6305
8
6
4
2
[ppm]



HPLC spectrum [$^{13}C_5$]C3G

50uM labelled ^{13}C-Labelled C3G (Matrix: <0.01%DMSO in 0.01% formic acid vv 100% methanol)

Acq. Instrument : Instrument 1 Location : P1-C-01
Injection Date : 4/1/2011 10:23:38 AM Inj : 1 Inj Volume : 5.0 µl
Acq. Method : C:\CHEM32\1\DATA\AM01\AM01_010411
Analysis Method : C:\CHEM32\1\DATA\AM01\AM01_010411
Method Info : AM01_pH differential of anthocyanins
Column: Eclipse XDB-C18 4.6 x 150 uM
Code: pn993967-902
Serial Number: USKH054260

Flow 1ml/min
A) 0.5% FA in H20 B) 0.5% Fa in MeOH
Gradient mins - %B
0 -2%
4 - 20%
6 - 80%
8 - 90%
10 - 100%
14 - 100%
15 - 0.5%
18 - 0.5%

==========

Area Percent Report

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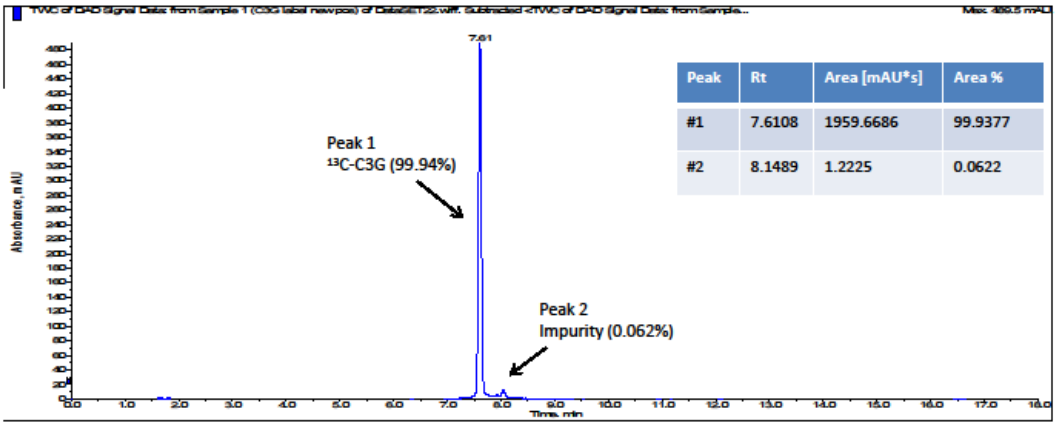
Signal 1: DAD1 A, Sig=265,10 Ref=700,10



Peak	Rt	Area [mAU*s]	Area %
#1	7.6108	1959.6686	99.9377
#2	8.1489	1.2225	0.0622

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*** End of Report ***