Electronic Supplementary Material (ESI) for Chemical Communications

Supporting information

NMR methodology for complex mixture 'separation'

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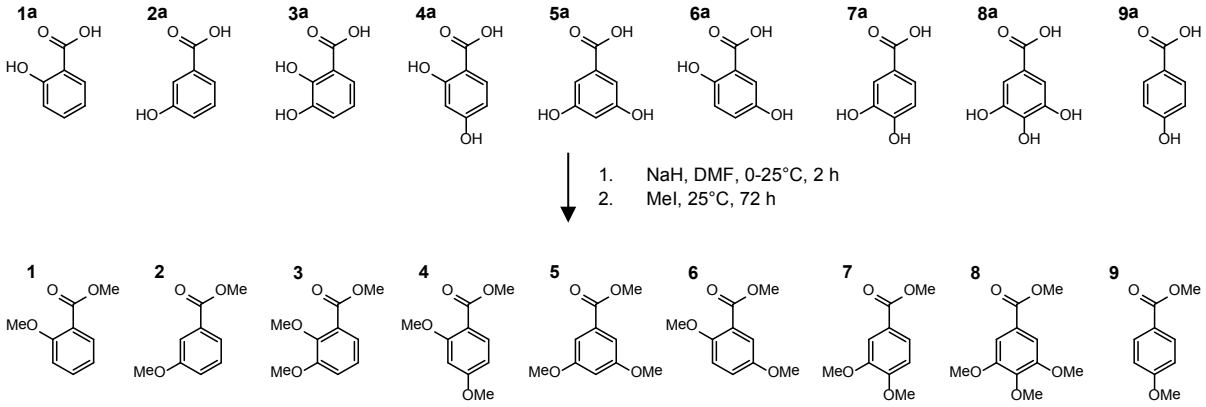
Materials and Methods

Methylation procedure.



Under nitrogen, sodium hydride 60% in oil (0.1420 g, 4.23 mmol) was washed with hexane (5 cm^3) and DMF (10 cm^3) added. The stirring suspension was cooled in an ice bath to 5 °C. A mixture of 2-hydroxybenzoic acid **1a** (0.0138 g, 0.10 mmol), 3-hydroxybenzoic acid **2a** (0.0138 g, 0.10 mmol), 2,3-dihydroxybenzoic acid **3a** (0.0154 g, 0.10 mmol), 2,4-dihydroxybenzoic acid **4a** (0.0154 g, 0.10 mmol), 3,5-dihydroxybenzoic acid **5a** (0.0154 g, 0.10 mmol), 2,5-dihydroxybenzoic acid **6a** (0.0154 g, 0.10 mmol), 3,4-dihydroxybenzoic acid **7a** (0.0154 g, 0.10 mmol), 3,4,5-trihydroxybenzoic acid **8a** (0.170 g, 0.10 mmol) and 4-hydroxybenzioc acid **9a** (0.0138 g, 0.10 mmol) was dissolved in DMF (5 cm^3) and the solution was added slowly to the stirring suspension of sodium hydride.

The mixture was warmed to room temperature and stirred for 2 h. Methyl iodide (0.2631 cm^3, 4.23 mmol) was added and stirred for a further 72 hours under reflux. The mixture was neutralised using 0.5 M HCl and water (30 cm^3) added. Chloroform (30 cm^3) was added, the water layer was separated and washed with chloroform (30 cm^3) and the organic fractions were combined. The combined organic fractions were washed with water (2 x 30 cm^3), dried over magnesium sulphate and evaporated (after removing chloroform, DMF was removed at 9 mbar @ 60 °C) to give a mixture of methyl (2-methoxy)benzoate **1**, methyl (3-methoxy)benzoate **2**, methyl (2,3-dimethoxy)benzoate **3**, methyl (2,4-dimethoxy)benzoate **4**, methyl (3,5-dimethyoxy)benzoate **5**, methyl (2,5-dimethoxy)benzoate **6**, methyl (3,4-dimethoxy)benzoate **7**, methyl (3,4,5-trimethoxy)benzoate **8** and methyl (4-methoxy)benzoate **9**.

800 µl of $CDCl_3$ were added to dissolve the compounds and the NMR sample was prepared by taking a few microliters from this concentrated solution to prepare a sample with an average concentration of 1.4 mM of **1-9** in 550 µl of $CDCl_3$. The NMR tube was sealed to avoid evaporation of $CDCl_3$ over time.

Experimental parameters of the 3D IPAP INEPT-INADEQUATE-HSQC experiment.

Details of the 3D IPAP INEPT-INADEQUATE-HSQC pulse sequence. The thin and thick bar represent 90° and 180° pulses, respectively, if not stated otherwise applied from the x axis, φ_1 = x,y, -x,-y; φ_2 = x; φ_3 = x (inphase) or y (antiphase spectra); ψ=x,-x. The following delays were used: $\Delta_1 = 1/4{}^1J_{CH}$, $\Delta_2 = 1/2{}^nJ_{CC}$. The shaded and blank PFGs were 1 and 0.5 ms long, respectively and were applied at G_1= 15%, G_2 = -15%, G_3= 15.06%, G_4= 18%, G_5= 9%, G_6= 5% and G_7= 70% of the maximum strength. The polarity of the G_1 and G_2 PFGs alternated for the collection of the echo/antiecho data. The φ_1 phase was incremented by 80° simultaneously with t_1 incrementation. STATES-TPPI protocol was applied to φ_2. The final two ^{13}C pulses were only applied when the inphase spectrum was acquired. The inset shows optional band selective (30 ppm) inversion pulses A and B applied at 80 and 30.0 ppm together with accompanying Bloch – Siegert shift (B.S.) compensating pulses.

Experimental parameters of the 3D IPAP INEPT-INADEQUATE-HSQC spectrum. Experiments were carried out at 15 °C on an 800 MHz Avance III (Bruker) NMR spectrometer equipped with a z-gradient triple-resonance TCI cryoprobe. The 3D IPAP INEPT-INADEQUATE-HSQC spectrum was acquired using t_1, t_2 and t_3 acquisition times of 10.6, 39.8 and 511 ms, respectively; Spectral widths of 75, 24 and 5 ppm were used. Four scans were acquired into each of 320 F_1 and 192 F_2 complex data points of each inphase and antiphase 3D spectra. The relaxation time was 1.4 s, resulting in the total experimental times of 2 days and 10 hours using 10% of non-uniform sampling. The $^1J_{CH}$ and $^nJ_{CC}$ used to calculate the Δ_1 and Δ_2 delays were 153 and 6 Hz respectively. Standard Bruker 0.5 and 2 ms adiabatic inversion and refocusing 180° ^{13}C pulses were used. The spectra were processed as described in the text, exponential multiplication (LB = 0.5 Hz), and cosine square window functions were used in F_3, F_2 and F_1, respectively. Zero filling (once) and linear prediction in F_1 and F_2 were used.

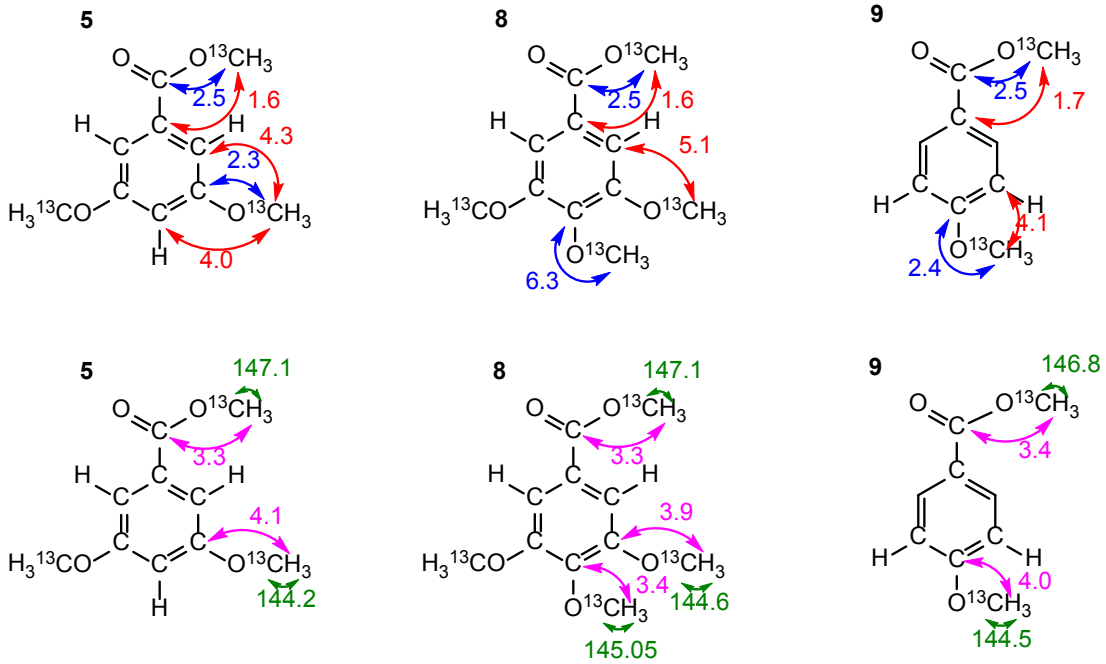


Figure S1. Carbon-carbon (top) and proton-carbon (bottom) coupling constants of compounds **5**, **8**, and **9** in Hz. Blue – $^2J_{CC}$, red – $^3J_{CC,}$ green – $^1J_{CH,}$ magenta – $^3J_{CH}$.

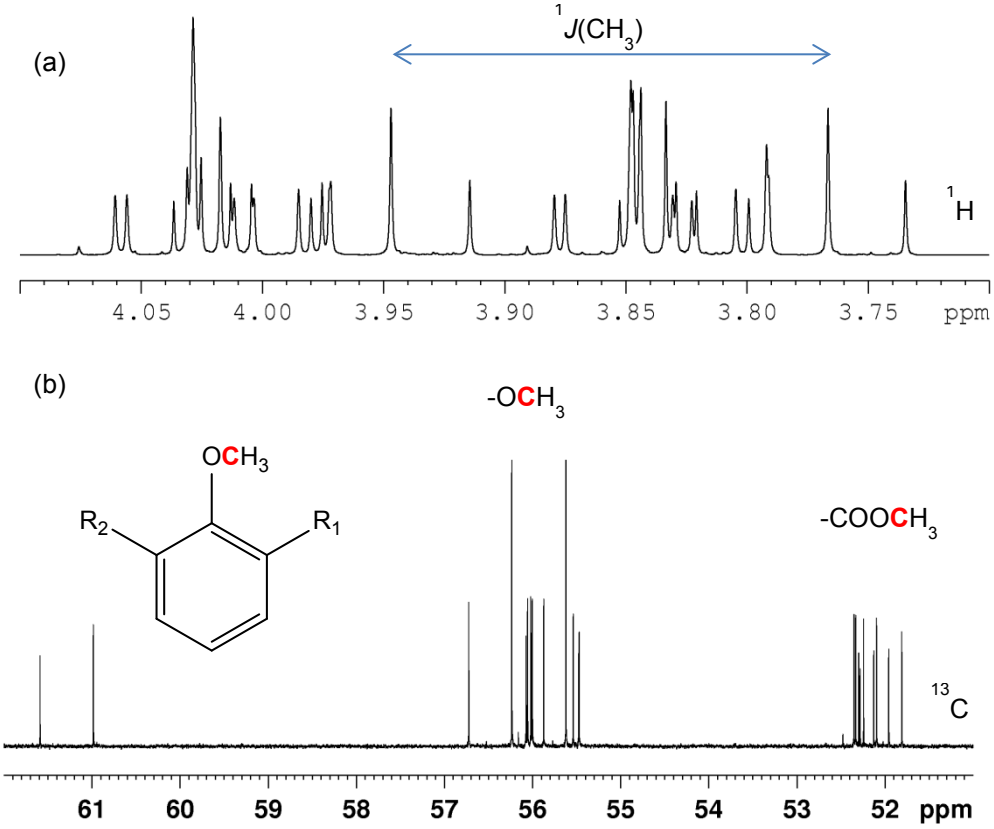


Figure S2. The methoxy regions of NMR spectra of the mixture of nine methylated compounds. (a) ^{13}C-coupled ^1H and (b) ^1H-decoupled ^{13}C spectra of the model mixture. While the proton chemical shifts are fairly insensitive to the -OCH_3 type, carbon chemical shifts show considerable differences. The different types are labelled in (b) (R_1, R_2 = OCH_3 or $COOCH_3$).

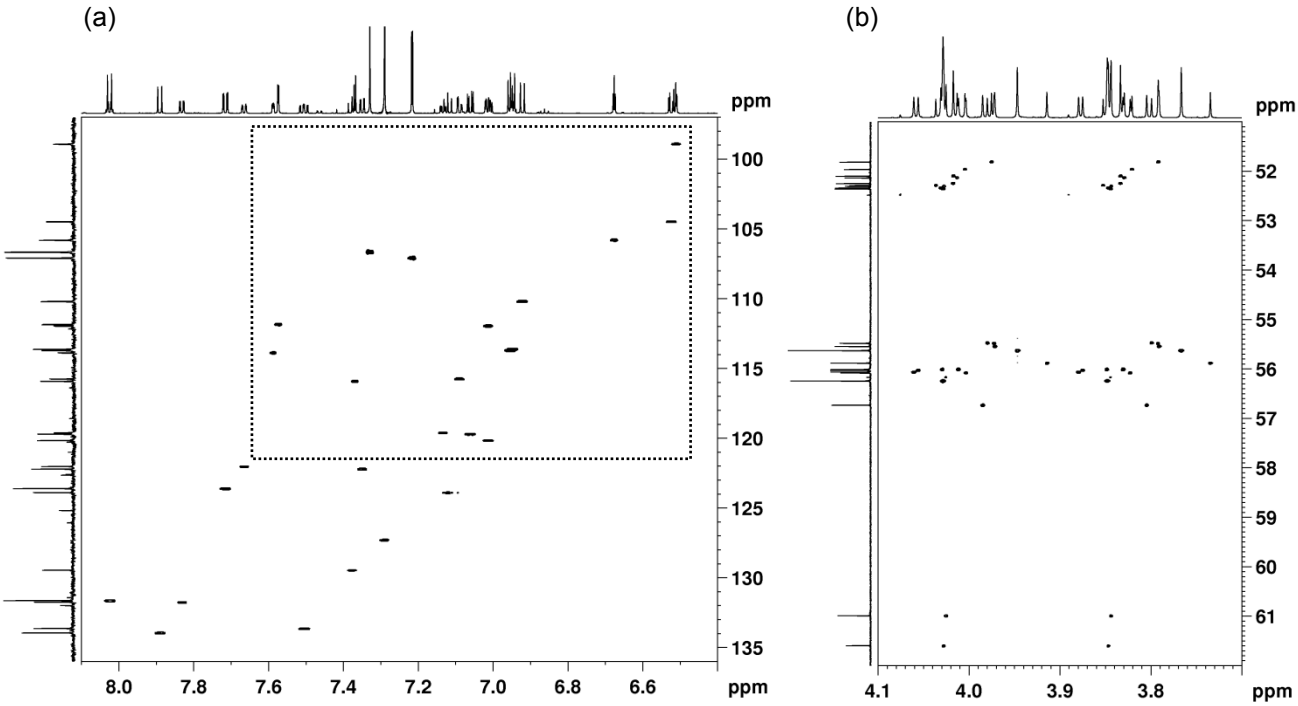


Figure S3. 2D ^1H, ^{13}C HSQC spectra of the model mixture (a) aromatic region acquired using the ^{13}C decoupling. The area enclosed in a dashed rectangle contains signals of nuclei ortho to -OCH_3 groups; (b) methyl region acquired without the ^{13}C decoupling. The t_1 and t_2 acquisition times (39.8 and 511 ms) were identical to corresponding acquistion times used in the 3D IPAP INEPT-INADEQUATE-HSQC experiment.

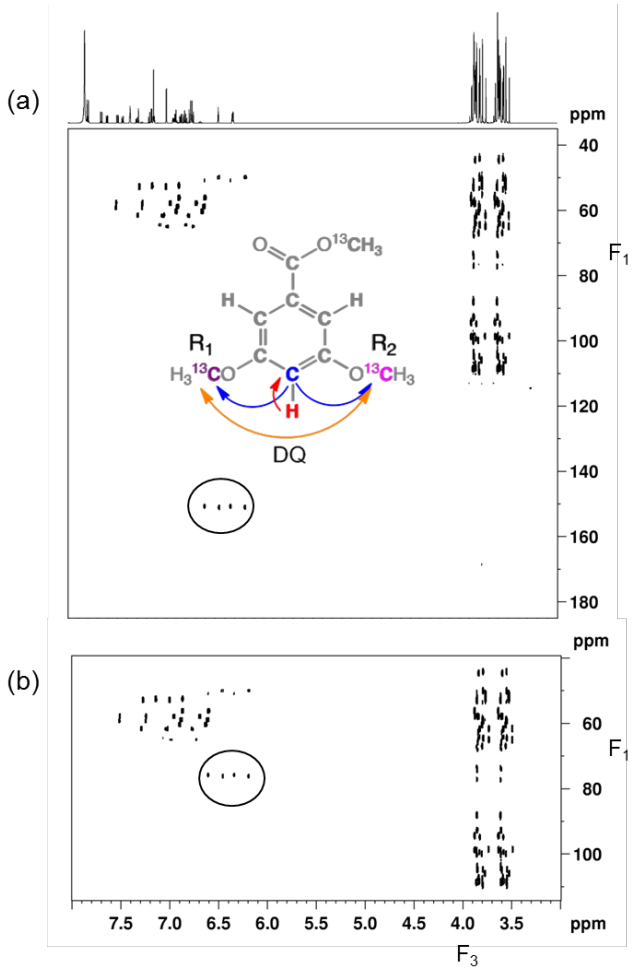


Figure S4. Optimising the spectral width of the DQ dimension. The first F_1F_3 DQ plane of the 3D IP INEPT-INADEQUATE-HSQC spectrum of the model mixture acquired without the ^{13}C decoupling. The ^{13}C carrier was set at 110 ppm; this value represents zero DQ frequency. The t_1 acquisition time was 2.8 ms. (a) SW1 was set to 150 ppm; no incrementation of the φ_1 phase (Fig. 1, main paper) was applied. (b) SW1 was set to 75 ppm and φ_1 was incremented by 80° every time the t_1 interval was increased. This represents the optimal sampling of the DQ dimension without the need for signal aliasing. An exception are the circled signals, which belong to DQ coherences created between the carbons of two -O^{13}CH$_3$ groups coupled to the same aromatic carbons as seen in molecules **4** and **5** shown in the inset. Their true DQ frequencies are ~ 1 ppm (2 x 55.5 – 110 ppm), hence they are aliased here. Their origin can be explained by following the evolution of magnetisation using the spin-product operators with reference to the pulse sequence of Fig. 1 and atom labelling of molecules **5** shown in (a).

$$I_z \xrightarrow{90°I_y} I_x \xrightarrow[2\pi J_{IS} 2\Delta_1 I_z S_z]{180°I_x, S_x} 2I_yS_z \xrightarrow{90°I_x, S_x} -2I_zS_y \xrightarrow[2\pi J_{SR1} 2\Delta_1 S_z R_{1z};\ 2\pi J_{SR2} 2\Delta_1 S_z R_{2z}]{180°S_x, R_{1x}, R_{2x}}$$

$$-8I_ZS_yR_{1z}R_{2z}\,sin(\pi J_{R1,S}2\Delta_2)\sin(\pi J_{R2,S}2\Delta_2) \xrightarrow{90°S_x, R_{1x}, R_{2x}} 8I_ZS_zR_{1y}R_{2y}$$

Followed by the back conversion to SQ ^{13}C coherences, refocusing of $^nJ_{CC}$ couplings and a reverse INEPT step, the $R_{1y}R_{2y}$ DQ coherences are detected on the aromatic proton I.

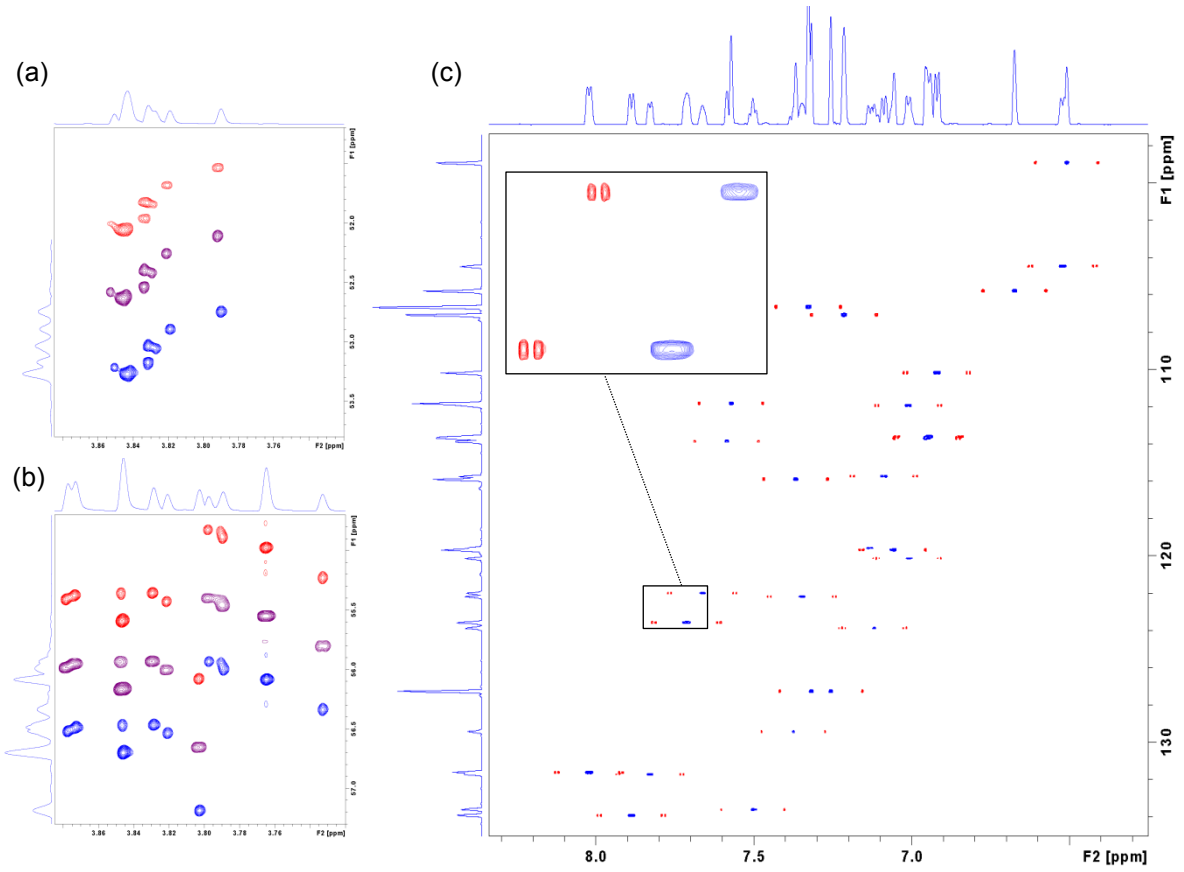


Figure S5. Processing of the ^{13}C-coupled 3D IPAP INEPT-INADEQUATE-HSQC spectra illustrated using the (a) -COO^{13}CH$_3$ region, (b) -O^{13}CH$_3$ region and (c) aromatic region of 2D IPAP HSQC spectra. (a) and (b) show an overlay of three spectra which were displaced vertically in order to separate the corresponding cross peaks. Blue peaks shows one half of the ^{13}CH$_3$ doublet from the 2D IP HSQC spectrum, purple and red cross peaks are the result of the following manipulation: inphase and antiphase spectra were added and subtracted generating interim spectra, which were shifted by $\pm^1J_{CH}$, resepcively, and added, restoring cross peaks at their chemical shift positions. For the purpose of this illustration they were moved in F_2 to coincide with the cross peaks of the IP spectrum and their vertical scales were halved. The purple spectrum was generated by setting $^1J_{CH}$ = 147.0 Hz, while the red spectrum is a result of using $^1J_{CH}$ = 144.6. It can be seen that the -COO^{13}CH$_3$ cross peaks (spectrum (a)) show the same resolution in the blue and purple spectrum, while the -O^{13}CH$_3$ cross peaks (spectrum (b)) show identical resolution in the blue and red spectrum. This is because the two types of -O^{13}CH$_3$ groups have different average values: $^1J_{COOCH3}$ = 147.05±0.21 Hz and $^1J_{OCH3}$ = 144.59±0.34 Hz. For optimal resolution, strip transformation is required using appropriate values of $^1J_{CH3}$. This is feasible due to the sepration of ^{13}C chemical shifts of -COO^{13}CH$_3$ and -O^{13}CH$_3$ groups (see Figure S2b).

The spread of the coupling constants of aromatic atoms ortho relative to -O^{13}CH$_3$ groups is larger ($^1J_{CH}$ = 160.8±1.9 Hz). The blue spectrum in (c) produced by signal centering therefore used only 1/3 of the t_3 time domain points (which corresponds to 170 ms acquisition time, i.e. the acquisition time typically used in in combination with ^{13}C decoupling on cryoprobes) and the sine bell square window function. This broadened the blue cross peaks, as illustrated in the inset, relative to those in the red 2D IP HSQC spectrum. Sensitivity was improved but the fine structure of aromatic proton multiplets was lost. Although it is feasible to shift each signal individually using appropriate value of $^1J_{CH}$ in this spectrum, this was not attempted since for more complex mixtures a wider range of structures and anticipated signal overlap may interfere with this process.

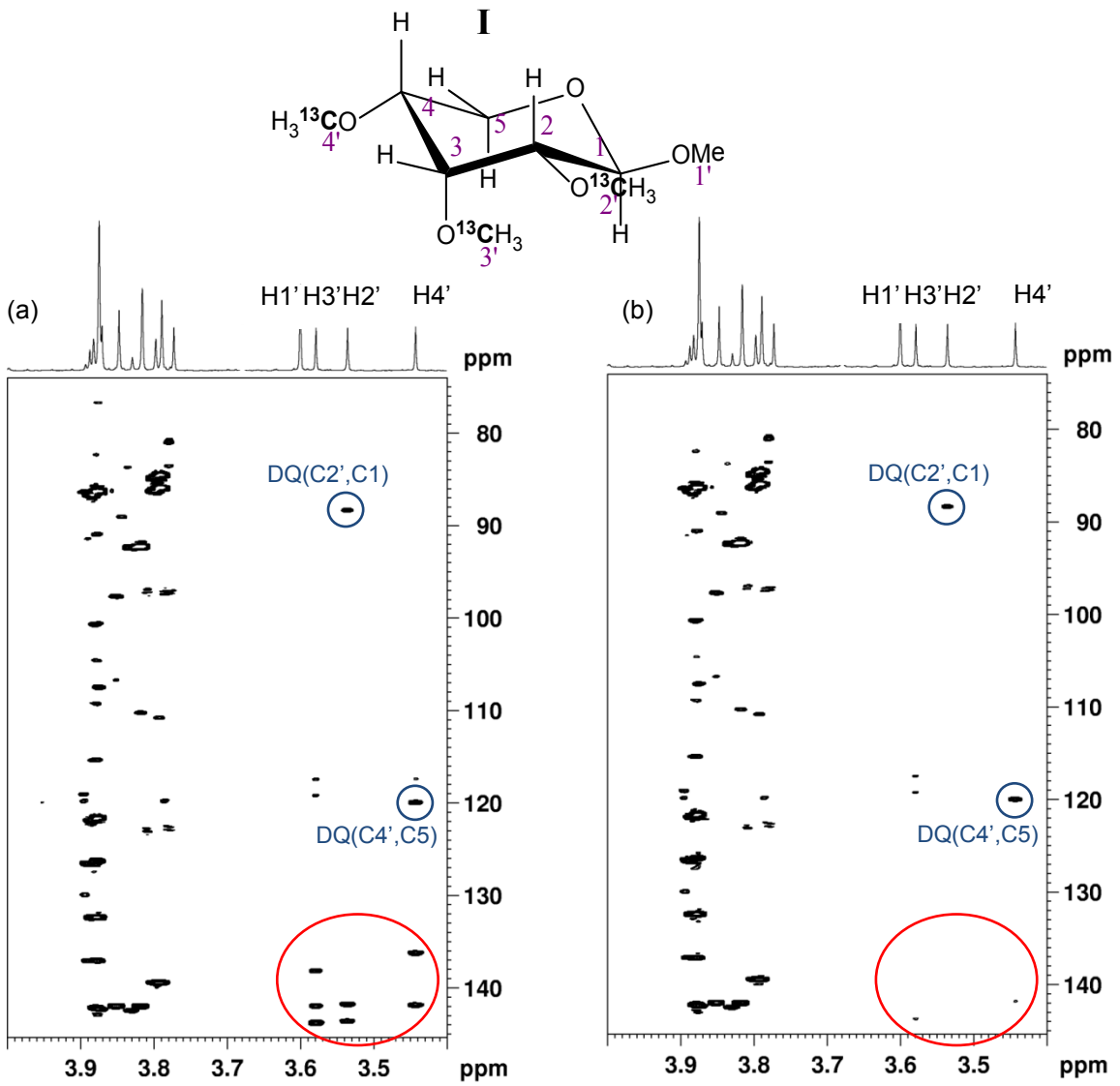


Figure S6. Illustration of the signal suppression from non-aromatic methylated compounds using a mixture of 7 compounds, including a ^{13}C methylated Me-β-D-xyloside, **I** (Me at C1 contains a $^{12}CH_3$ group). The carbon chemical shifts of **I** are 104.6 (C1), 84.9 (C3), 83.1 (C2), 79.3 (C4) and 63.0 (C5). (a) and (b) show the methyl region of the first DQ plane of the 3D IPAP INEPT-INADEUQATE-HSQC experiment with no suppression and with the suppression of aliphatic resonances, respectively. The red circle highlights the signals that were suppressed by the procedure. Their residual signal intensity in the suppressed spectrum was 6 ± 4% of the original signals. The C2'C1 and C4'C5 DQ coherences highlighted in blue were not suppressed at all, as C1 and C5 chemical shifts are outside of the inversion band of the 1.5 ms IPURP-2 pulses applied at 80 and 30 ppm. The intensity of the DQ coherences involving the aromatic OMe carbons (cross peaks on the left side of the spectra) were not affected at all.

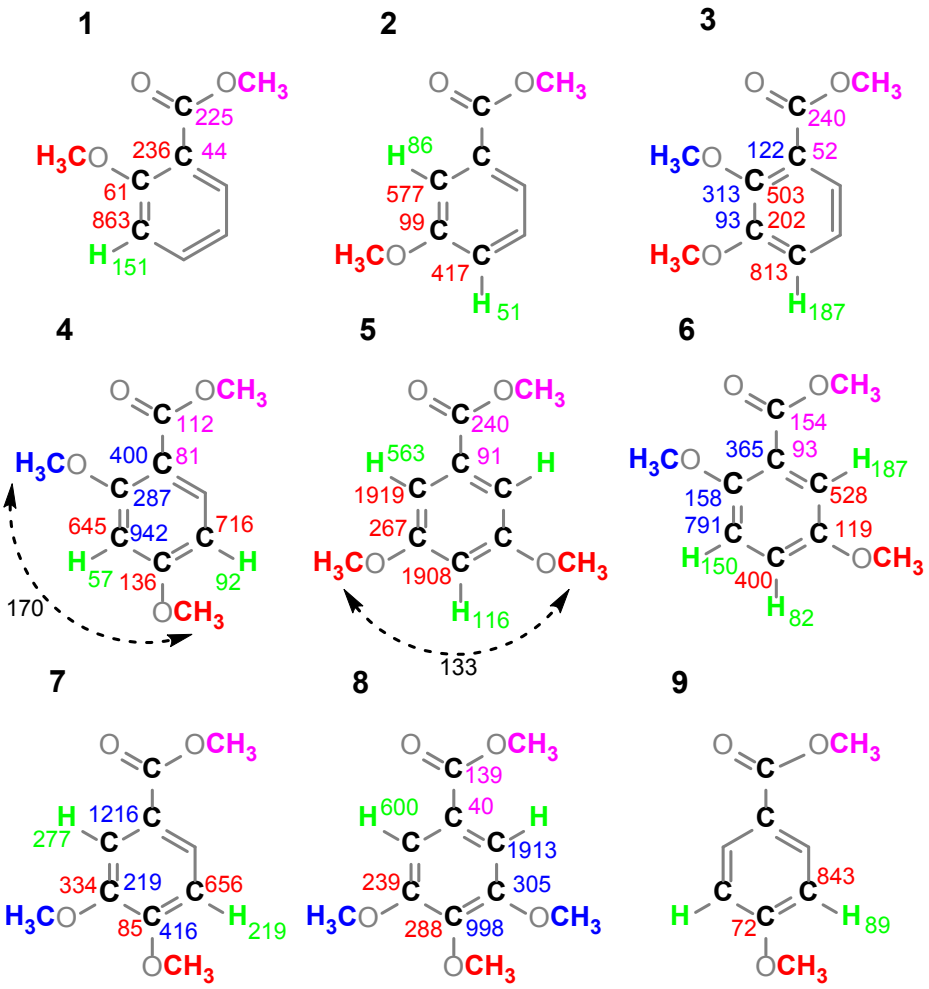


Figure S7. S/N ratios obtained from F_3 1D traces extracted from F_1F_3 planes of a 3D spectrum obtained by the addition of the IP and AP 3D IPAP INEPT-INADEQUATE-HSQC spectra. The magenta, blue and red colours are used to link the -OCH_3 signal and the carbons forming the DQ coherence for which the S/N is given. The green label is used for the S/N of the aromatic protons corresponding to the DQ coherence between their bonded carbon and the -OCH_3 carbon in the ortho position. The black labels and the dashed arrows indicate the S/N of the DQ coherences between two meta -OCH_3 groups observed on the aromatic proton positioned between them. See Figure S4 for the explanation of their origin.