

# *J*-modulated ADEQUATE (JM-ADEQUATE) experiment for accurate measurement of carbon–carbon coupling constants

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## Abstract

A new method for the accurate determination of carbon–carbon coupling constants is described. The method is based on a modified ADEQUATE experiment, where a *J*-modulated spin-echo sequence precedes the ADEQUATE pulse scheme. The *J*-modulation and scaling of carbon–carbon couplings is based on simultaneous incrementation of <sup>13</sup>C chemical shift and coupling evolution periods. The time increment for the homonuclear carbon–carbon coupling evolution can be suitably scaled with respect to the corresponding increment for the chemical shift evolution. Typically a scaling factor of 2 to 3 is employed for the measurement of one-bond coupling constants, while multiplication by a factor of 10 to 15 is applied when small long-range couplings are determined. The same pulse scheme with coupling evolution period optimized for one-bond or long-range couplings allows the determination of the corresponding carbon–carbon coupling constants. The splittings of the ADEQUATE crosspeaks in the F<sub>1</sub> dimension yield the appropriately multiplied coupling constants.

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## 1. Introduction

Homonuclear <sup>13</sup>C–<sup>13</sup>C chemical shift correlation experiments provide direct connectivity information about the skeleton of organic molecules. These experiments are particularly important when other methods fail to provide unambiguous structural data. The low natural abundance of the <sup>13</sup>C isotope (1.1%) gives rise to ca. 0.01% probability of two <sup>13</sup>C nuclei being connected to each other through one or more bonds; the majority (0.99%) of <sup>13</sup>C spins are isolated and/or coupled to <sup>1</sup>H, which makes the homonuclear <sup>13</sup>C–<sup>13</sup>C correlation experiments difficult to carry out.

Early experiments, such as the traditional INADEQUATE [1–11] schemes use the evolution of carbon–carbon double quantum coherence followed by carbon detection, and the undesired background carbon magnetization of <sup>12</sup>C–<sup>13</sup>C isotopomers is suppressed by

appropriate phase cycling. The inherent low sensitivity of these experiments, however, prevents their routine use, especially when only limited sample amount is available. Recently, a sensitivity enhanced version of carbon detected INADEQUATE with composite refocusing (INADEQUATE CR) [9,12–14] and off-resonance compensation [8] has been devised to improve the sensitivity. One-dimensional selective variants [15,16] have also been introduced and applied successfully to decrease the number of correlations and shorten the experimental time. One-dimensional experiments have an additional advantage in providing accurate coupling constant values for structural analysis. The introduction of pulsed field gradients in different proton- and carbon detected INADEQUATE experiments provide spectra with superior parent signal suppression, moreover the simultaneous detection of equivalent coherence pathways (PEP) in echo–antiecho scheme leads to improved sensitivity. The sensitivity can be further enhanced by employing heteronuclear polarization transfer steps (INEPT, DEPT, reverse INEPT) [17,18] in

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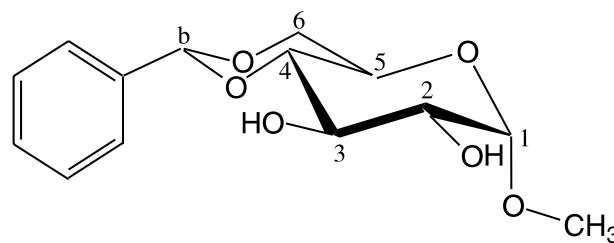
the preparation block of the sequence, but thereby restricting the observable correlations only to protonated carbons.

Recently proposed carbon–carbon correlation experiments [19–23] (ADEQUATE) combine the evolution of carbon–carbon double quantum ( $^{13}\text{C}$ – $^{13}\text{C}$  DQ) and carbon single quantum ( $^{13}\text{C}$  SQ) coherence employing heteronuclear correlations through one-, or multiple bonds with HSQC, HMQC or HMBC schemes to detect  $^1\text{H}$  magnetization and so achieve the highest accessible sensitivity. Note that the effective sensitivity improvement of the  $^1\text{H}$ -detected experiment depends on the proton multiplet structure, since the intensity is distributed on the relevant resonances. Although the HMBC scheme extends the applicability of the method to quaternary carbons, the long preparation period results in significant signal loss due to  $T_2$  relaxation.

In addition to detection of carbon–carbon chemical shift correlation, accurate determination of  $^{13}\text{C}$ – $^{13}\text{C}$  coupling constants (one-, two-, and three-bond) is also of central importance, because these parameters carry structural information that are not available from any other data [24–32].

## 2. Results and discussion

The experiment proposed here uses the ADEQUATE principle preceded with a carbon–carbon  $J$ -modulation spin-echo scheme to assess the desired carbon–carbon coupling constants (Fig. 1). 4,6-Benzylidene-1-methoxy- $\alpha$ -D-glucose (**1**) (Scheme 1) was used as a model compound to illustrate the utility of the experiment for the



4,6-benzylidene-1-methoxy- $\alpha$ -D-glucose

Scheme 1. Formula of (**1**).

measurement of both one-bond and long-range carbon–carbon couplings. The sequence starts with an INEPT polarization transfer to enhance the carbon sensitivity. Then a  $J$ -modulation spin-echo sequence is inserted with a weighted ( $sc$ , scaling) [33] spin-echo delay to enhance the effect of  $J$ -modulation by scaling the value of the actual increment. The next  $90^\circ$   $^{13}\text{C}$ -pulse pair with opposite phase and separated with a  $z$ -gradient pulse purges the antiphase  $^{13}\text{C}$  magnetization ( $\sin(\pi J_{CC} sc t_1)$ ) and preserves only the in-phase component ( $\cos(\pi J_{CC} sc t_1)$ ). This part of the sequence results in doublet splitting of the crosspeaks along the  $F_1$  dimension of the ADEQUATE spectrum. The frequency separation of doublet lines provides the actual  $^{13}\text{C}$ – $^{13}\text{C}$  coupling constant upscaled by the applied scaling factor ( $sc$ ). The value of  $sc$  is chosen according to the magnitude of the carbon–carbon coupling constant; the typically used scaling factor is  $sc = 3$  for one-bond and  $sc = 15$  for long-range correlations, respectively. The applied  $sc$  values enhance the separation within the

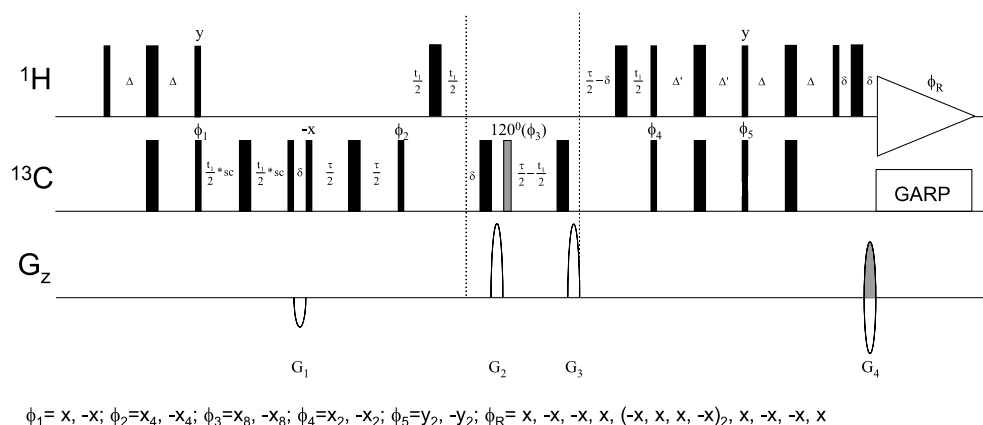


Fig. 1. Pulse sequence of the  $J$ -modulated ADEQUATE (JM-ADEQUATE) experiment. Narrow and wide bars stand for nonselective  $90^\circ$  and  $180^\circ$  pulses, respectively. The grayed pulse corresponds to  $^{13}\text{C}$   $120^\circ$ . Note that all  $^{13}\text{C}$   $180^\circ$  pulses can be replaced by smoothed chirp pulses to avoid off-resonance effects, as proposed by Köck et al. [35] Echo–antiecho selection is achieved by the pulsed field gradients:  $G_2 = 78.4\%$ ,  $G_3 = 77.4\%$ , and  $G_4 = \pm 59\%$ , given as percentage of the absolute gradient strength of 50 G/cm.  $G_1 = -31\%$  is used for purging. Gradient lengths are 1 ms followed by a recovery delay of 200  $\mu\text{s}$ .  $\Delta$  is set to  $^1J_{\text{CH}} = 155$  Hz.  $\tau$  is adjusted to the corresponding one-bond ( $^1J_{\text{CC}}$ ) or multiple-bond ( $^nJ_{\text{CC}}$ ) coupling constant. Also the multiplication factor ( $sc$ ) is chosen according to the type of carbon–carbon correlation. The two spectra shown in Figs. 2 and 3 were recorded with  $sc = 3$  and  $sc = 15$ , respectively. Phase cycling is depicted under the pulse scheme.  $G_4$  and  $\phi_5$  are inverted in every other experiment for echo–antiecho selection.

doublet, however, the effective resolution enhancement achieved depends on the line width as well [33], which is also scaled depending upon the transverse relaxation rates of the relevant single and multiple quantum coherences. The  $J$ -modulation block is followed by carbon–carbon coupling evolution of constant duration ( $\tau$ ), where the value of  $\tau$  determines the type of correlation. Accordingly,  $\tau$  is optimized to the magnitude of one-bond or long-range carbon–carbon coupling ( $\tau = 1/(2^1J_{CC})$  or  $1/(2^nJ_{CC})$ ), in case of direct or remote correlations, respectively. The rest of the pulse scheme is the same as in the original ADEQUATE experiment. So as carbon–carbon double quantum coherence is generated by the subsequent  $^{13}\text{C}$   $90^\circ$  pulse. During the first incremented delay ( $t_1$ ) period DQ frequency labeling occurs. The  $120^\circ$   $^{13}\text{C}$  pulse with the subsequent spin-echo and carbon–proton back transfer sequence creates and refocuses the detectable proton single-quantum coherence. The net effect of the two incremented delay periods leads to frequency modulation, equivalent to the single quantum frequency of the carbon bound to the INEPT excited protonated carbon.

Fig. 2 shows the two-dimensional JM-ADEQUATE spectrum of **1** optimized for one-bond  $^{13}\text{C}$ – $^{13}\text{C}$  correlations (45 Hz) and using a scaling factor ( $sc$ ) of 3 to enhance the separation of doublet lines in the  $F_1$  dimension for the measurement of the desired carbon–carbon couplings. The expanded region with correlations of the sugar residue is presented. One-bond

correlations were obtained for all  $^{13}\text{C}$ – $^{13}\text{C}$  spin pairs (C1–C6). Carbons 2, 3, 4, and 5 have two neighbors so the corresponding proton signals (H2–H5) provide two crosspeaks each along the  $F_1$  dimension, while H1, H6a, and H6b display only one correlation to C2 and C5 resonances, respectively. The splitting of the  $F_1$  doublets, as shown in the selected traces, provide the desired one-bond carbon–carbon coupling constants upscaled by a factor of three. Note that this correlation scheme allows a duplicate measurement of the coupling constant for each interaction, e.g.,  $^1J_{C2,C3}$  can be determined from both H3/C2 and H2/C3 doublets. The digital resolution of 0.9 Hz per point achieved in  $F_1$  after zero-filling allowed an accurate measurement of the one-bond coupling constants. The experimental details are given in the legend of Fig. 2.

Homonuclear multiple-bond  $^{13}\text{C}$ – $^{13}\text{C}$  coupling constants have also been measured with the proposed JM-ADEQUATE sequence optimized for 4 Hz ( $^nJ_{CC}$ ) and with a scaling factor ( $sc$ ) of 15 as shown in Fig. 3. Expansions of the JM-ADEQUATE spectrum showing all multiple-bond correlations of the benzylidene carbon (Cb) of **1** is presented. The two- and three-bond coupling constants are measured from the line separation of doublets that is upscaled by the applied multiplication factor of 15. A digital resolution of 0.2 Hz per point attained after zero-filling in  $F_1$  was suitable for measuring the long-range coupling constants. It should be noted, however, that if the doublets are not well resolved

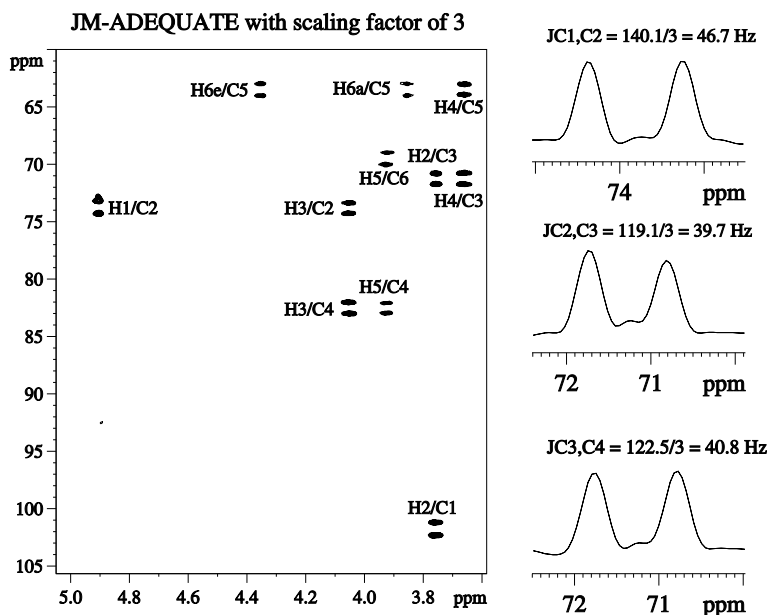


Fig. 2. JM-ADEQUATE spectrum of **1** displaying  $F_1$ -doublets for one-bond carbon–carbon coupling constant measurement. The spectrum was obtained with the pulse sequence depicted in Fig. 1 using a scaling factor ( $sc$ ) of 3 to enhance the separation of doublet lines. One-bond coupling constants measured on selected correlations (H1/C2, H2/C3, and H4/C3) are shown on the corresponding  $F_1$ -traces. The delays,  $\Delta$  and  $\tau$ , were set to the corresponding coupling constant of 155 Hz ( $^1J_{CH}$ ) and 45 Hz ( $^1J_{CC}$ ). A relaxation delay of 1.8 s was allowed between subsequent transients. The spectrum was acquired with 320  $t_1$  increments and 64 scans leading to 24 h measuring time. Zero-filling in  $F_1$  to 2K real data points and apodization with shifted squared sine-bell function in both dimensions were applied prior to Fourier transformation.

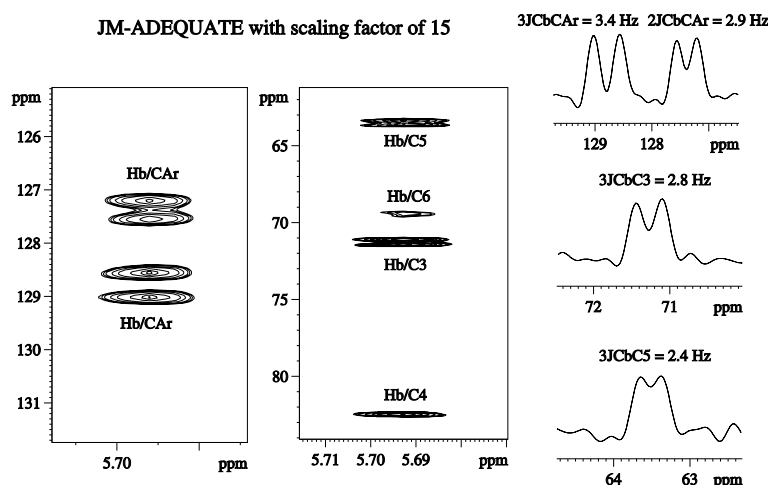


Fig. 3. Parts of JM-ADEQUATE spectrum of **1** displaying  $F_1$ -doublets for multiple-bond carbon-carbon coupling constant measurement. The spectrum was recorded with the pulse sequence shown in Fig. 1 using a scaling factor ( $sc$ ) of 15 to multiply the separation of doublet lines. Two- and three-bond coupling constants measured on selected correlations (Hb/CAr, Hb/C3, and Hb/C5) are shown on the corresponding  $F_1$ -traces. The delays,  $\Delta$  and  $\tau$ , were adjusted to the corresponding coupling constant of 155 Hz ( $^1J_{CH}$ ) and 4 Hz ( $^nJ_{CC}$ ). A relaxation delay of 1.8 s was allowed between subsequent transients. The spectrum was acquired with 320  $t_1$  increments and 128 scans leading to 48 h experiment time. Zero-filling in  $F_1$  to 2K real data points and apodization with shifted squared sine-bell in  $F_2$  and Gaussian window function in  $F_1$  dimension were applied prior to Fourier transformation.

(e.g., see Hb/C5 correlation), the measured couplings are underestimates of their true value. In principle, the separation of the doublet lines could be further improved by increasing the applied scaling factor, however, the magnetization loss due to  $T_2$  relaxation during the extended  $J$ -modulation period sets an upper limit of the choice of  $sc$ .

Finally, the *accuracy* of the coupling constant measurement from in-phase vs. antiphase doublets is compared and illustrated in case of one-bond carbon-carbon coupling in Fig. 4. Since the coupling constant is measured in the incremented dimension, it is crucial to provide high spectral resolution with properly chosen number of  $t_1$  increments for the accurate reading. The long experiment time and  $T_2$  relaxation loss, however, put restriction on the number of the acquired points in  $F_1$ . Fig. 4 demonstrates that the superior separation of

the in-phase doublet lines obtained with the proposed JM-ADEQUATE experiment using a scaling factor of 3 makes the coupling constant measurement fairly insensitive to the number of  $t_1$  increments varying that from 200 to 500. To the contrary, in case of antiphase doublets (Fig. 4B) obtained with the modified 1,1-ADEQUATE experiment [21], the separation of antiphase lines varies [34], and accordingly an oscillation in the value of the relevant coupling constant can clearly be seen with increasing  $t_1$  increments. Of course, as was shown before [34], accurate values for the coupling can be obtained by appropriate fitting procedure.

### 3. Experimental

All experiments were performed on a Bruker Avance DRX 500 spectrometer (Bruker GmbH, Germany) equipped with a broad-band inverse (BBI) z-gradient probe. All spectra were processed with XWINNMR 2.6 (Bruker GmbH, Germany). The sample was prepared by dissolving ca. 200 mg of (**1**) in 600  $\mu$ l of DMSO- $d_6$ /C $_6$ D $_6$  solvent mixture. After transferring the solution in NMR tube, the tube was sealed. The temperature was set to 313 K for all measurements.

All  $J$ -multiplied ADEQUATE spectra were acquired with suitably chosen spectral resolution allowing accurate measurement of the desired one-bond or long-range coupling constants. Length of  $90^\circ$  pulse was 9.8 and 13.0  $\mu$ s for  $^1H$  and  $^{13}C$ , respectively. Note that  $^{13}C$   $180^\circ$  rectangular pulses can be replaced by smoothed chirp pulses [35–37] to avoid off-resonance effects [38]. GARP decoupling with  $^{13}C$   $90^\circ$  pulse of 75  $\mu$ s was applied

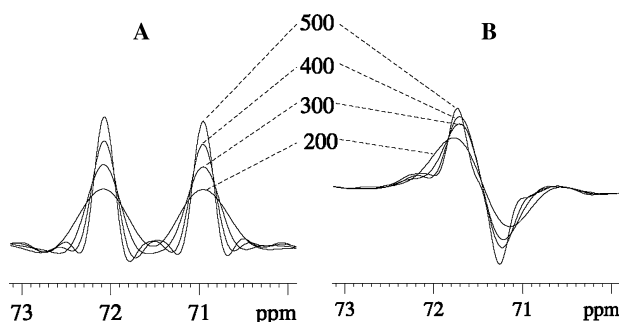


Fig. 4. Illustration of the dependence of the accuracy of coupling constant measurement on the number of  $t_1$  increments. Coupling constant is determined from the in-phase (A) and the antiphase (B) doublets. In the former case a scaling factor of 3 was applied for increasing the separation of doublet lines.

during acquisition. Experimental details of the measurements are summarized in the relevant figure legends.

#### 4. Conclusion

Carbon–carbon coupling constants at natural abundance of the  $^{13}\text{C}$  nucleus can be accurately measured by means of a modified ADEQUATE sequence extended with  $J$ -modulated spin-echo period which allows simultaneous evolution of carbon–carbon coupling and DQ/SQ coherence. Summarizing the advantages of the proposed sequence: (i) allows simple extraction of coupling constants by measuring frequency difference between peak maxima of in-phase doublets, (ii) allows accurate measurement of both the large one-bond and the small long-range carbon–carbon couplings with appropriate choice of the experimental parameters, (iii) allows  $J$ -multiplication, consequently the spectral resolution in  $F_1$  (i.e., the number of  $t_1$  increments) can be reduced without degrading the accuracy of the coupling constant measurement.

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