

Proton-Detected ^{13}C – ^{13}C Double-Quantum Coherence

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One of the most successful applications of multiple-quantum NMR spectroscopy is the INADEQUATE experiment (1–3) that in a very straightforward manner traces out the carbon skeleton of molecules at the natural-abundance level of ^{13}C . Often the 2D pulse sequence is tuned to only one-bond J_{CC} coupling constants which results in a spectrum showing correlations for all pairs of covalently bound ^{13}C atoms, but also long-range correlations provide valuable structural information.

Whereas the natural-abundance level compared to full ^{13}C enrichment has the advantage of spectral simplicity, there is a severe penalty in terms of sensitivity. That has led to a number of approaches for improving the sensitivity of the natural-abundance INADEQUATE experiment. Sørensen *et al.* (4), Sparks and Ellis (5), and Podkorytov (6) introduced combinations with INEPT (7, 8) and DEPT (9)-type polarization transfer from ^1H to ^{13}C while Keller and Vogele (10) chose to detect ^1H magnetization by doing the opposite polarization transfer at the end of the INADEQUATE pulse sequence. However, only the addition of pulsed-field gradients (PFG) that greatly ease suppression of the intense ^1H signals stemming from molecules with less than two ^{13}C atoms has made ^1H -detected INADEQUATE (11–14) feasible on a routine basis. The latest technique, INADEQUATE CR (15–17), returned to ^{13}C detection and about doubles the sensitivity compared to the original approaches (1–6).

With reference to the theory described by Ernst *et al.* (18), Refs. (13, 15) discussed the relative sensitivity of ^1H - and ^{13}C -detected INADEQUATE. This Communication has nothing to add to that discussion. Instead, we take ^1H detection and PFG for suppression of undesired coherence-transfer pathways for granted and introduce new, and compare with existing, pulse sequences for ^1H -detected INADEQUATE. In fact, the most useful new experiment is in its simple form possible only with ^1H detection. It will be shown that the choice of pulse sequence depends on the prior information available about the molecule. For convenience we distinguish two classes of sequences: those where coherence

transfer between proton and carbon spins is effected via one-bond or long-range J_{CH} couplings, respectively.

For $\text{C} \rightarrow \text{H}$ transfers via $^1J_{\text{CH}}$ couplings, we distinguish four different types of pulse sequences. First, there is the option of whether to refocus carbon magnetization antiphase with respect to $^1J_{\text{CH}}$ prior to excitation of ^{13}C – ^{13}C double-quantum coherence {2QC}. If both carbons of a ^{13}C – ^{13}C pair are protonated, and especially for CH groups, refocusing can be advantageous, because intensity loss into ^{13}C – ^{13}C zero-quantum coherence is minimized; this can represent up to a factor of two in sensitivity. On the other hand, there is nothing to gain by refocusing when one of the carbons is quaternary. For sequences with or without refocusing, we recommend DEPT- or INEPT-type $\text{C} \rightarrow \text{H}$ transfers, respectively.

Another option is whether to attempt coherence-order-selective (COS) transfer (19–24) from ^{13}C – ^{13}C {2QC} to detectable ^1H {–1QC}, i.e., designing the pulse sequence so as to emphasize one of the transfers $\{+2\text{QC}\}^{\text{CC}} \rightarrow \{-1\text{QC}\}^{\text{H}}$ or $\{-2\text{QC}\}^{\text{CC}} \rightarrow \{-1\text{QC}\}^{\text{H}}$ at the expense of the other. If that is possible and sufficiently efficient, it is worthwhile in connection with PFG to, independent of the pulse sequence, eliminate one of the pathways. PFG are essential for detection of ^{13}C – ^{13}C satellites in proton spectra because of the need for efficient suppression of signals from molecules without, or with only a single, ^{13}C nucleus.

Combining the two sets of binary options, we arrive at the pulse sequences outlined in Figs. 1a–1d. For $\text{H}_n\text{C}^{13}\text{CH}_m$ spin systems, the signal detected for the protons of the CH_n group in the $t_1 = 0$ 1D spectrum of the 2D experiment has the following intensities for the various sequences relative to those of a corresponding 1D spectrum recorded after a single $\pi/2$ pulse (neglecting relaxation, pulse imperfections, and ^1H – ^1H couplings):

$$\text{Fig. 1a: } f_{\text{H}_n\text{CCH}_m}^{\text{DEPT2}} = \frac{1}{4} s_{\theta_1} (n c_{\theta_1}^{n-1} + m c_{\theta_1}^{m-1}) s_{\theta_2} c_{\theta_2}^{n-1} \quad [1]$$

$$\text{Fig. 1b: } f_{\text{H}_n\text{CCH}_m}^{\text{INEPT2}} = \frac{1}{4} \quad [2]$$

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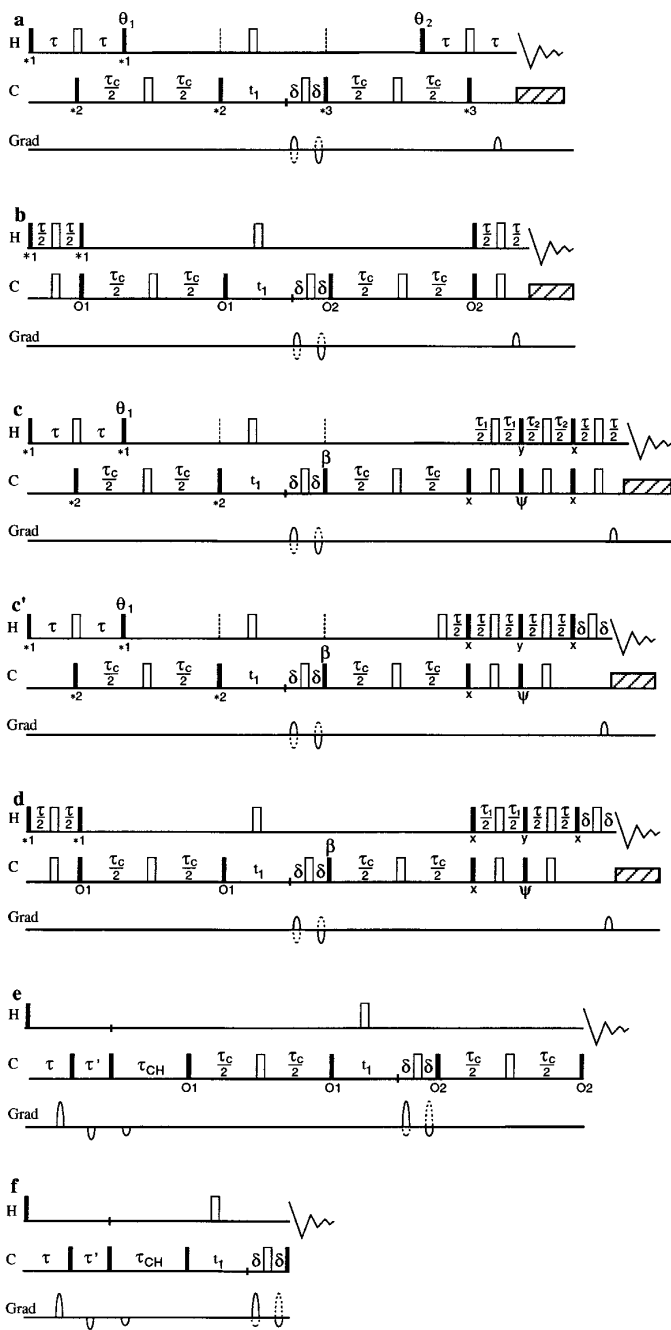


FIG. 1. ^1H -detection pulse sequences (a) DEPT2, (b) INEPT2 (13), (c) DEPT-COS, (c') DEPT-COS (optimized for H^{13}C - ^{13}CH and ^{13}C - ^{13}CH segments), (d) INEPT-COS (14), and (e) HMBC INADEQUATE, and (f) 2Q HMBC. Unless specified otherwise above the pulses, filled and open bars refer to $\pi/2$ and π pulses, respectively. The dashed lines indicate optional purging pulses (25) at points where the desired components of the density operator are inphase with respect to $^1J_{\text{CH}}$. τ_{C} is the INADEQUATE delay tuned according to J_{CC} , $\tau = (2^1J_{\text{CH}})^{-1}$, and τ_{CH} is the HMBC delay tuned according to $^nJ_{\text{CH}}$. The sequences in (e) and (f) include a (second-order) low-pass J filter (26). Pulsed-field gradients are, as indicated, of relative amplitudes 1, 2, or 3. The full-line gradients apply for echo selection whilst the two gradients following t_1 must be reversed for anti-echo selection (dashed lines). In combination with the gradients, the COS versions differ in the settings of β (27) and ψ (22, 23) according to echo $\{\beta = \pi/3, \psi$

$$\text{Fig. 1c: } f_{\text{H}_n\text{CCH}_m}^{\text{DEPT-COS}} = \frac{3\sqrt{3}}{32} s_{\theta_1} (nc_{\theta_1}^{n-1} + mc_{\theta_1}^{m-1}) \times c_2^{n-1} (s_1 c_1^{n-1} + s_2) \quad [3]$$

$$\text{Fig. 1d: } f_{\text{H}_n\text{CCH}_m}^{\text{INEPT-COS}} = \frac{3\sqrt{3}}{32} (s_1 + c_1^{n-1}). \quad [4]$$

These equations assume $\tau = (2^1J_{\text{CH}})^{-1}$, $\tau_{\text{C}} = (2J_{\text{CC}})^{-1}$, and use the abbreviations $c_{\theta}^r = \cos^r(\theta)$, $s_{\theta}^r = \sin^r(\theta)$, $c_q^p = \cos^p(\pi^1J_{\text{CH}}\tau_q)$, and $s_q^p = \sin^p(\pi^1J_{\text{CH}}\tau_q)$. Transfer efficiencies and pulse sequence parameters for pulse sequences tuned for optimum transfer for H_nCCH_m spin systems with $n = 1, 2$, or 3 and $m = 0, 1, 2, 3$ are given in Table 1. The expressions in Eqs. [1]–[4] and the numbers in Table 1 apply irrespective of whether J_{CC} is a one-bond or a long-range coupling constant.

With the understanding that any experiment is only as good as the intensity of the weakest signal of interest in the resulting spectrum, it is evident from Table 1 that the experiment of choice for general applications is INEPT2 INADEQUATE. The exception is when the only protonated carbons are of the CH type, in which case the COS option is worthwhile. For H^{13}C - ^{13}CH segments, DEPT-COS INADEQUATE theoretically has a factor of two sensitivity advantage over the corresponding INEPT-based experiment, whereas both experiments exhibit the same sensitivity for ^{13}C - ^{13}CH segments. Even when CH_2 and CH_3 groups are present, the COS option favoring CH groups can be advantageous because, particularly in aliphatic systems, the ^1H signals of CH groups are often the weakest.

A final comment on the $^1J_{\text{CH}}$ -based pulse sequences in Figs. 1a–1d is that these sequences miss ^{13}C - ^{13}C pairs when both carbons are nonprotonated. In order to pick up these quaternary–quaternary pairs, it is necessary to either employ long-range ^1H - ^{13}C couplings for the heteronuclear coherence transfers (14) or to use INADEQUATE in its original form (1–3) with ^{13}C detection and NOE enhancement, or preferably in the CR version (15–17).

An HMBC-based (29) pulse sequence with a few modifications compared to that presented by Reif *et al.* (14) is shown in Fig. 1e. Note that there is no worthwhile COS version of this experiment: the detected signals have, in contrast to the sequences in Figs. 1c, 1c', and 1d, a simple sine dependence on the flip angle of the ^{13}C pulse after the second δ delay. A low-pass J filter (26) eliminates magneti-

$= -y$ or $\beta = 2\pi/3, \psi = y$ for echo, and $\{\beta = \pi/3, \psi = y$ or $\beta = 2\pi/3, \psi = -y$ for anti-echo. Two pulses with an asterisk below them must have a relative phase displacement of $-\pi/2$ or $\pi/2$, whereas those with a circle below them must have 0 or π . Finally, the ^{13}C parts of the sequences (a)–(e) are of the symmetrical type allowing uniform excitation of 2QCs (28). A pulse sequence similar to that in (f) has been developed independently by Reif *et al.* (32).

TABLE 1
Amplitudes for $F_z + G_z \rightarrow 2S_1^+ S_2^+ \rightarrow F^-$ Transfer in H_n CCH $_m$ Spin Systems ($F = \sum_{i=1}^n I_i$, $G = \sum_{j=1}^m I_j$, $I = {}^1H$, $S = {}^{13}C$)
Using the 1H -Detected INADEQUATE Pulse Sequences in Figs. 1a–1d^a

H \rightarrow {CC} \rightarrow H transfer	n m	1				2				3			
		0	1	2	3	0	1	2	3	0	1	2	3
DEPT2 ^b		0.250 ^{c,d}	0.500 ^{c,d}	0.440 ^{d,e}	0.444 ^{d,f}	0.125 ^{g,h}	0.220 ^{e,h}	0.250 ^{g,h}	0.265 ^{h,i}	0.111 ^{j,k}	0.171 ^{j,k}	0.204 ^{i,k}	0.222 ^{j,k}
<i>c,d,t</i>		0.250	0.500	0.250	0.250	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>g,h,m</i>		0.125	0.250	0.302	0.313	0.125	0.213	0.250	0.258	0.094	0.156	0.182	0.188
<i>j,k,n</i>		0.083	0.167	0.219	0.250	0.111	0.179	0.222	0.247	0.111	0.167	0.202	0.222
INEPT2 ^p		0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250
DEPT-COS ^b		0.325 ^{c,p}	0.650 ^{c,p}	0.572 ^{c,p}	0.577 ^{f,p}	0.143 ^{g,q}	0.252 ^{e,q}	0.286 ^{g,q}	0.303 ^{i,q}	0.125 ^{j,r}	0.192 ^{j,r}	0.229 ^{i,r}	0.250 ^{j,r}
<i>c,l,p</i>		0.325	0.650	0.325	0.325	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>g,m,q</i>		0.149	0.299	0.360	0.373	0.143	0.244	0.286	0.294	0.106	0.176	0.205	0.211
<i>j,n,r</i>		0.098	0.195	0.257	0.293	0.127	0.204	0.254	0.282	0.125	0.187	0.227	0.250
INEPT-COS ^b		0.325 ^s	0.325 ^s	0.325 ^s	0.325 ^s	0.230 ^t	0.230 ^t	0.230 ^t	0.230 ^t	0.203 ^u	0.203 ^u	0.203 ^u	0.203 ^u
<i>l,s</i>		0.325	0.325	0.325	0.325	0.162	0.162	0.162	0.162	0.162	0.162	0.162	0.162
<i>m,t</i>		0.277	0.277	0.277	0.277	0.230	0.230	0.230	0.230	0.196	0.196	0.196	0.196
<i>n,u</i>		0.244	0.244	0.244	0.244	0.222	0.222	0.222	0.222	0.203	0.203	0.203	0.203

^a $J = {}^1J_{CH}$, $\tau = 0.5/J$.

^b Optimized for the actual n , m values.

^c $\theta_1 = 90^\circ$.

^d $\theta_2 = 90^\circ$.

^e $\theta_1 = 53.62^\circ$.

^f $\theta_1 = 41.81^\circ$.

^g $\theta_1 = 45^\circ$.

^h $\theta_2 = 45^\circ$.

ⁱ $\theta_1 = 38.87^\circ$.

^j $\theta_1 = 35.26^\circ$.

^k $\theta_2 = 35.26^\circ$.

^l Optimized for $n = 1$.

^m Optimized for $n = 2$.

ⁿ Optimized for $n = 3$.

^o Valid for all n , m values.

^p $\tau_1 = \tau_2 = 0.5/J$.

^q $\tau_1 = 0.25/J$, $\tau_2 = 0.20/J$.

^r $\tau_1 = 0.20/J$, $\tau_2 = 0.15/J$.

^s $\tau_1 = 0.5/J$.

^t $\tau_1 = 0.25/J$.

^u $\tau_1 = 0.1667/J$.

zation from protons directly attached to ${}^{13}C$ nuclei as these contributions are more conveniently exploited in the pulse sequences in Figs. 1a–1d.

The HMBC-INADEQUATE experiment in Fig. 1e yields a 2D spectrum with peak intensities proportional to $\sin^2\{\pi J_{CC}\tau_C\} \sin\{\pi({}^nJ_{CH} + {}^{n'}J_{CH})\tau_{CH}\}$ and, as pointed out by Reif *et al.* (14), this expression vanishes for ${}^nJ_{CH}/{}^{n'}J_{CH} \approx -1$ which is not uncommon for $n = 2$, $n' = 3$. Another case of vanishing peak intensity is for J_{CC} close to zero which can occur for long-range J_{CC} , in particular over two or four bonds.

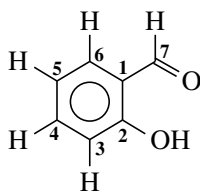
Our solution to the above-mentioned two pitfalls of HMBC INADEQUATE is the pulse sequence in Fig. 1f, which we dub 2Q HMBC. In this experiment, the ${}^{13}C$ – ${}^{13}C$

2QCs are excited directly from 1H 1QCs rather than via ${}^{13}C$ 1QCs as in the other experiments of Fig. 1. The peak intensities in 2Q HMBC are therefore independent of J_{CC} and proportional to $\sin\{\pi{}^nJ_{CH}\tau_{CH}\} \sin\{\pi{}^{n'}J_{CH}\tau_{CH}\}$.

Typically, 2Q HMBC and HMBC-INADEQUATE spectra will have a few peaks in common but otherwise be quite complementary. Peaks associated with vanishing of either ${}^nJ_{CH}$ or ${}^{n'}J_{CH}$ can show up in HMBC INADEQUATE, while they will be very weak or missing entirely in 2Q HMBC. On the other hand, peaks associated with $J_{CC} \approx 0$ are unique for 2Q HMBC; in particular, ${}^2J_{CC}$ and ${}^4J_{CC}$ correlations can be very strong when ${}^nJ_{CH}$ and ${}^{n'}J_{CH}$ both are three-bond coupling constants. Furthermore, 2Q HMBC is short, simple, and rather insensitive to strong coupling between carbons,

which can be a problem for INADEQUATE-type excitation of 2QC (30).

For experimental confirmation, salicylaldehyde was chosen



because it is an example of ${}^n J_{\text{CH}}/{}^{n'} J_{\text{CH}} \approx -1$. This holds true for the coupling constants between the hydroxyl proton and the C1 and C2 carbons, which in benzene solution are 4.60 and -4.77 Hz, respectively (31). The DEPT-COS INADEQUATE experiments (Figs. 1c and 1c') were in all our attempts inferior to the INEPT-based sequence (Fig. 1d) for pairs of quaternary and methine carbons. We have not inves-

tigated this discrepancy between theory and experiment in detail but it appears that the DEPT-based COS sequences are only worthwhile for the special case where the methine signals of $\text{CH}-\text{CH}_m$ pairs must have highest possible intensity.

In Fig. 2 are shown HMBC ${}^1 J_{\text{CC}}$ -INADEQUATE, HMBC ${}^n J_{\text{CC}}$ -INADEQUATE, and 2Q HMBC spectra. As expected, the C1-C2 peak detected via the hydroxyl proton is missing in HMBC INADEQUATE and clearly visible in 2Q HMBC; the C2-C3 peak is similar in this respect [values in benzene (31) are $J_{\text{C2OH}} = -4.77$ Hz and $J_{\text{C3OH}} = 7.37$ Hz]. In addition, the 2Q HMBC spectrum shows strong peaks excited via ${}^3 J_{\text{CH}}$ couplings (i.e., associated with pairs of carbons separated by two or four bonds) and a number of other long-range correlations. Some of these are visible in the HMBC ${}^n J_{\text{CC}}$ -INADEQUATE spectrum. However, the $J_{\text{CC}} \approx 0$ peaks unique for 2Q HMBC are absent altogether. It is further noteworthy that the 2Q HMBC spectrum shows correlations based on small ${}^4 J_{\text{CH}}$ coupling constants [values in benzene (31) are $J_{\text{C2H5}} = -1.39$ Hz and $J_{\text{C3H6}} = -1.32$ Hz].

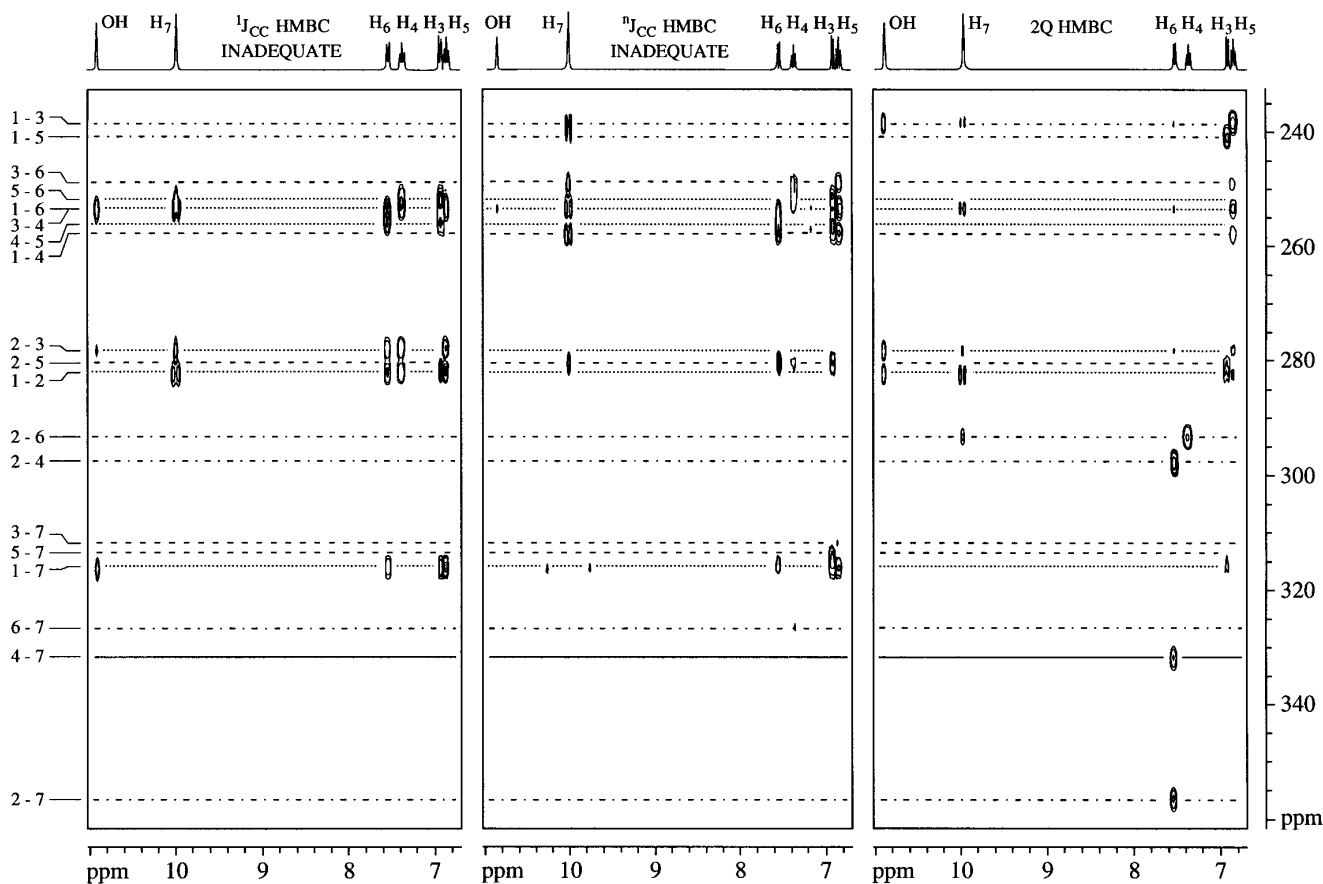


FIG. 2. HMBC INADEQUATE and 2Q HMBC spectra of salicylaldehyde dissolved in DMSO-d_6 (0.5 ml in 0.2 ml) recorded on a Bruker 400 MHz AMX-2 spectrometer. All spectra shown were recorded at 300 K and the spectral widths covered were 15094 and 2203 Hz in ω_1 and ω_2 , respectively. Parameters: $\tau = 3.114$ ms, $\tau' = 2.834$ ms, $\tau_{\text{CH}} = 58.798$ ms, $\tau_{\text{C}} = 8.9286$ ms/62.5 ms; prescan delay 4 s; number of scans 64. Time-domain data matrices of 128×1024 points were zero-filled to 256×1024 prior to Fourier transformation, with window functions cosine squared and sinebell shifted by $\pi/4$ in ω_1 and ω_2 , respectively. Dotted, dot-dashed, dashed, and full lines refer to one-, two-, three-, and four-bond ${}^{13}\text{C}-{}^{13}\text{C}$ correlations.

In conclusion, we have analyzed four different pulse sequences for detection of ^{13}C – ^{13}C double-quantum coherence via protons and with heteronuclear coherence transfers mediated by $^1J_{\text{CH}}$ couplings. For most practical applications, the sequence of choice is that combining INEPT and INADEQUATE [Ref. (13) and Fig. 1b] while only special cases with full prior knowledge of the numbers of protons attached to carbons and consideration of intensities in the ^1H spectrum justifies more elaborate pulse sequences [Ref. (14) and Figs. 1c, 1c', 1d]. For the class of experiments based on heteronuclear coherence transfers mediated by long-range J_{CH} couplings, we have optimized a pulse angle in the sequence presented in Ref. (14) (Fig. 1e) and introduced a novel experiment, 2Q HMBC (Fig. 1f) that provides additional and complementary structural information because the peak intensities, in contrast to all previous experiments, are independent of J_{CC} .

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