

Communication

HAT HMBC: A hybrid of H2BC and HMBC overcoming shortcomings of both

Andrew J. Benie, Ole W. Sørensen *

Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-2500 Valby, Denmark

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Abstract

A new 2D NMR experiment, HAT HMBC, that is a hybrid of H2BC and HMBC aims at establishing two-bond correlations absent in H2BC spectra because of vanishing ${}^3J_{\text{HH}}$ coupling constants. The basic idea is to create an additional π phase difference in the multiplet structure in HMBC peaks with respect to the ${}^{n+1}J_{\text{HH}}$ coupling constant between the proton(s) attached to a ${}^{13}\text{C}$ and a ${}^1\text{H}$ separated by n bonds. Thus HMBC peaks associated with small J_{HH} will be the most attenuated in a HAT HMBC spectrum in comparison to a regular HMBC spectrum, i.e. peaks associated with ${}^{n+1}J_{\text{HH}}$ and ${}^nJ_{\text{CH}}$ will for $n > 2$ usually be strongly attenuated. The HAT HMBC pulse sequences contain the same number of pulses as regular HMBC and are only a few milliseconds longer.

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Heteronuclear ${}^{13}\text{C}$ – ${}^1\text{H}$ long-range NMR correlation is crucial in structure determination of small- and medium-size molecules at natural abundance isotope levels. Such spectra serve to link the pairs of directly bonded ${}^1\text{H}$ and ${}^{13}\text{C}$ nuclei identified in a one-bond ${}^1\text{H}$ – ${}^{13}\text{C}$ spectrum along with long-range correlations to quaternary carbons. The HMBC [1] experiment yields the largest number of long-range correlations as the key coupling constant for the correlation is that between the ${}^1\text{H}$ and ${}^{13}\text{C}$ nuclei being correlated, which results in correlations over mainly two and three bonds but occasionally also over four or more rarely even five bonds.

Whereas a great number of long-range correlations on one hand is desirable, it comes with the problem of assigning the correlations with respect to the number of bonds between the ${}^1\text{H}$ and ${}^{13}\text{C}$ spins being correlated. An HMBC spectrum usually does not contain this kind of information. Another limitation of HMBC is that by virtue of the coherence transfer mechanism there is no correlation peak when

the underlying long-range J_{CH} coupling constant vanishes, which occurs in some cases for two-bond J_{CH} s.

H2BC [2,3] is another long-range correlation experiment aimed at remedying the above two shortcomings of HMBC. For protonated ${}^{13}\text{C}$ nuclei it circumvents the problem of vanishing ${}^2J_{\text{CH}}$ coupling constants by using another coherence transfer based on ${}^1J_{\text{CH}}$ and ${}^3J_{\text{HH}}$ coupling constants for generation of ${}^1\text{H}$ – ${}^{13}\text{C}$ two-bond correlation peaks. There are two main benefits in this context, namely (i) correlation peaks are observed independent of ${}^2J_{\text{CH}}$ when the corresponding ${}^3J_{\text{HH}}$ coupling constant does not vanish, which typically yields some peaks not present in the HMBC spectrum, (ii) identification of the correlations over two bonds. The latter point is quite reliable in H2BC spectra although it must be kept in mind that unusually large ${}^4J_{\text{HH}}$ coupling constants can lead to three-bond correlation peaks in H2BC spectra. In general, HMBC and H2BC spectra are complementary and both are recommended in routine applications.

This paper addresses and proposes a remedy for a gap left by H2BC and HMBC spectra, namely that two-bond correlation peaks are weak or absent in H2BC spectra if ${}^3J_{\text{HH}}$ is small or vanishes. Such peaks can be observed in

* Corresponding author. Fax: +45 3327 4765.

E-mail address: olews@crc.dk (O.W. Sørensen).

HMBC spectra relying on ${}^2J_{\text{CH}}$ that does not necessarily vanish when ${}^3J_{\text{HH}}$ does. However, in an HMBC spectrum the identification as a two-bond correlation is not usually obvious. Therefore, we introduce a new experiment aimed at identifying two-bond correlations in HMBC-type spectra.

In the H2BC experiment, the critical ${}^{n+1}J_{\text{HH}}$ coupling constant enters twice in the intensity expression of a correlation peak, namely in the coherence transfer function (as $\sin\{\pi^{n+1}J_{\text{HH}}T\}$) and by virtue of the peak being antiphase with respect to this coupling constant in the acquisition dimension of the experiment. For the same correlation peak in an HMBC spectrum the same holds true but with ${}^{n+1}J_{\text{HH}}$ replaced by ${}^nJ_{\text{CH}}$ and T by Δ . Our new experiment is based on HMBC but ideally introduces an additional π relative phase difference between the two submultiplets representing the two possible spin states of each proton directly attached to the ${}^{13}\text{C}$ of an HMBC correlation peak. If the pertinent ${}^{n+1}J_{\text{HH}}$ is small relative to the inverse of the HMBC excitation delay Δ , the result is mainly antiphase character with respect to ${}^{n+1}J_{\text{HH}}$ in the acquisition dimension suggesting the name Homonuclear J ATenuated (HAT) HMBC. Hence, in comparison with an HMBC spectrum, correlation peaks will be absent in a HAT HMBC spectrum when ${}^{n+1}J_{\text{HH}}$ vanishes. This is similar to an H2BC spectrum and thus a HAT HMBC spectrum shows predominantly two-bond correlations. However, as for H2BC, this holds true only for protonated ${}^{13}\text{C}$ nuclei and not for quaternary carbons as for these there is no ${}^{n+1}J_{\text{HH}}$ to attenuate the peaks.

There is an earlier experiment by Sprang and Bigler [4] aiming to achieve the same end as HAT HMBC. However, the intensity expression for a peak in their spectrum is essentially the product of the corresponding H2BC and HMBC intensity expressions as both ${}^{n+1}J_{\text{HH}}$ and ${}^nJ_{\text{CH}}$ enter twice. In other words, all peaks in a Sprang–Bigler two-bond spectrum will be weaker than in a corresponding H2BC spectrum, and such a spectrum will not show peaks that are absent in the latter. Another advantage of HAT HMBC (Fig. 1) is the simplicity of the pulse sequences as they contain the same number of pulses as regular HMBC and only are prolonged by a few milliseconds. A more elaborate modification of HMBC aiming at distinguishing two- and three-bond correlations based on tilts of correlation peaks in a single spectrum has also been described [5].

Clearly, no peak will be detected for ${}^1\text{H}-\{{}^{13}\text{C}-\text{H}_m\}$ moieties in a HAT HMBC spectrum when the J_{HH} coupling constants are extremely small. However, there is a range of “small” ${}^3J_{\text{HH}}$ coupling constants where the sensitivity of HAT HMBC is significantly better than the sensitivity of H2BC due to the absence of $\sin\{\pi^{n+1}J_{\text{HH}}T\}$ in the coherence transfer function in HAT HMBC. It can even happen that the intensity of two-bond correlation peaks is higher in the HAT HMBC than in the regular HMBC spectrum due to the change in phases by the “hatting” procedure. For example, for peaks where the excitation delay Δ is on the order of $({}^{n+1}J_{\text{HH}})^{-1}/2$ a ${}^{n+1}J_{\text{HH}}$ antiphase

character in HMBC is converted to an inphase character in HAT HMBC, which, everything else equal, is beneficial for the sensitivity.

The HAT HMBC experiment is outlined in Fig. 1 in two different pairs of versions. The additional element in comparison to the HMBC pulse sequence is that the heteronuclear zero- and double-quantum coherences undergo evolution under the one-bond J coupling constant during a $({}^1J_{\text{CH}})^{-1}/2$ delay introduced between the two $\pi/2(\text{C})$ pulses surrounding t_1 yielding an additional π phase difference of multiplet components. The $\pi/2$ evolution under ${}^1J_{\text{CH}}$ of the individual ${}^1J_{\text{CH}}$ doublet components can be in the positive or the negative sense, which represents the difference between the versions in Fig. 1a and b that otherwise are equivalent. We refer to the two experiments of a HAT HMBC pair as HAT– and HAT+ with the sign indicating whether the $\pi({}^{13}\text{C})$ pulse is applied before or after the $\pi({}^1\text{H})$ pulse, respectively.

Addition or subtraction of the HAT– and HAT+ data sets allow editing into two subspectra according to the number of protons attached to the ${}^{13}\text{C}$ being even (i.e. C and CH_2 groups) or odd (i.e. CH and CH_3 groups).

As mentioned above, the aim of the HAT HMBC pulse sequence pair is to impose $e^{i\phi}$ and $e^{-i\phi}$ phase factors on the two submultiplets of each proton attached to a ${}^{13}\text{C}$ nucleus where $\phi = (\pi^1J\tau)$ ideally is equal to $\pi/2$. The real part of such a phase factor represents the component that is unaffected by the “hatting” operation whilst the imaginary part represents the “hatted” component. It is the “hatted” components that are targeted in the even/odd editing, and the “unhatted” components are responsible for J cross talk.

This feature of HAT HMBC spectra leads to an unusual situation for multiplicity-edited spectra. Usually, if there is J cross talk, i.e. a signal in a wrong subspectrum, there will also be a signal at the same position in the correct subspectrum. HAT HMBC is an exception to this rule because there can be a peak in the wrong subspectrum and nothing at all in the correct one, which is easy to recognize. This can occur when the pertinent ${}^{n+1}J_{\text{HH}}$ vanishes, because the antiphase character across ${}^{n+1}J_{\text{HH}}$ suppresses the “hatted” components in the correct subspectrum while the “unhatted” component inphase with respect to ${}^{n+1}J_{\text{HH}}$ ends up in the wrong subspectrum. It is a firm rule of HAT HMBC spectra that peaks in the wrong subspectrum for the respective carbons should be disregarded. They show up because of a deviation $\Delta(^1J)$ between the actual 1J and 1J_0 used in setting the $\tau/2$ delays and hence contain no information about pertinent J_{HH} coupling constants.

Editing into the two HAT HMBC subspectra is not necessary for the “hatting” effect as that is present in both experiments of a HAT HMBC pair of pulse sequences, but the editing is necessary to separate out the unhatted components, and it does not change the sensitivity. Furthermore, and similar to edited HMBC spectra [6] but in contrast to edited H2BC spectra [3], possible residual one-bond correlation peaks unsuppressed by the low-pass J filter [7] show up in the wrong subspectrum for the

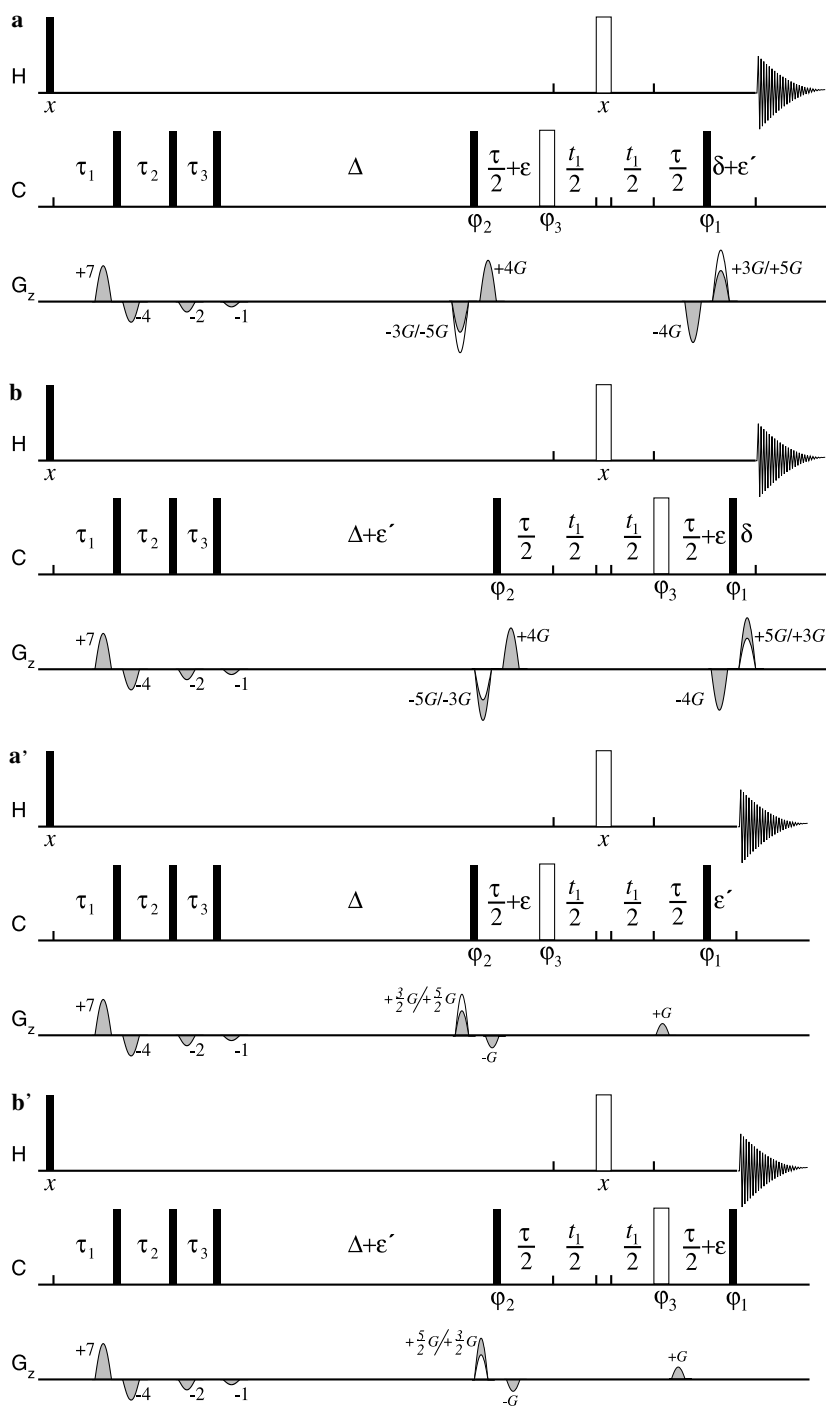


Fig. 1. HAT HMBC pulse sequences with a third order low-pass J filter and editing into two subspectra according to the number of directly attached protons being odd or even: filled and open bars refer to $\pi/2$ and π pulses, respectively, while the dashed open boxes represent ^{13}C decoupling. $\tau = (2 \ ^1J_{\text{CH}})^{-1}$ and δ is the delay necessary for a gradient, ϵ is equal to twice the minimum $t_1/2$ time plus the time taken for a proton π pulse, ϵ' is the same as ϵ but with the addition of the time needed for a carbon π pulse. The initial four gradients of the low-pass J filter can be set an order of magnitude weaker than the gradients used for formation of heteronuclear gradient echoes. The delays for the third order low-pass J filter are $\tau_1 = 1/2[{}^1J_{\text{min}} + 0.07({}^1J_{\text{max}} - {}^1J_{\text{min}})]^{-1}$, $\tau_2 = [{}^1J_{\text{max}} + {}^1J_{\text{min}}]^{-1}$, $\tau_3 = 1/2[{}^1J_{\text{max}} - 0.07({}^1J_{\text{max}} - {}^1J_{\text{min}})]^{-1}$. The recommended phase cycle is an even number out of $\varphi_1 = \{x, -x, -x, x\}$, $\varphi_2 = \{x, x, 4(-x), x, x\}$, $\varphi_3 = \{4(x), 4(y), 4(-x), 4(-y)\}$ with the receiver phase always alternating between x and $-x$. (a) HAT-HMBC pulse sequence where the echo is selected by the $-3\text{G}/+3\text{G}$ gradients and the antiecho by the $-5\text{G}/+5\text{G}$ gradients. (b) HAT+HMBC pulse sequence effecting $\Pi/2$ evolutions under ${}^1J_{\text{CH}}$ in the opposite sense to the HAT-sequence; the echo is selected by the $-5\text{G}/+5\text{G}$ gradients and the antiecho by the $-3\text{G}/+3\text{G}$ gradients. Preferably the data of the two pulse sequences should be recorded in an interleaved manner. After formation of the required two linear combinations in the time domain the data are processed in the same way as other HMBC-type data [12]. (a') and (b') are alternatives to (a) and (b), respectively, with the advantage of being a gradient delay shorter but with the disadvantage of dephasing by gradients across the t_1 period. With the typical experimental parameters for small molecules no difference in performance has been observed between the (a, b) and (a', b') versions. Pulse programs for HAT HMBC can be downloaded from www.crc.dk/nmr.

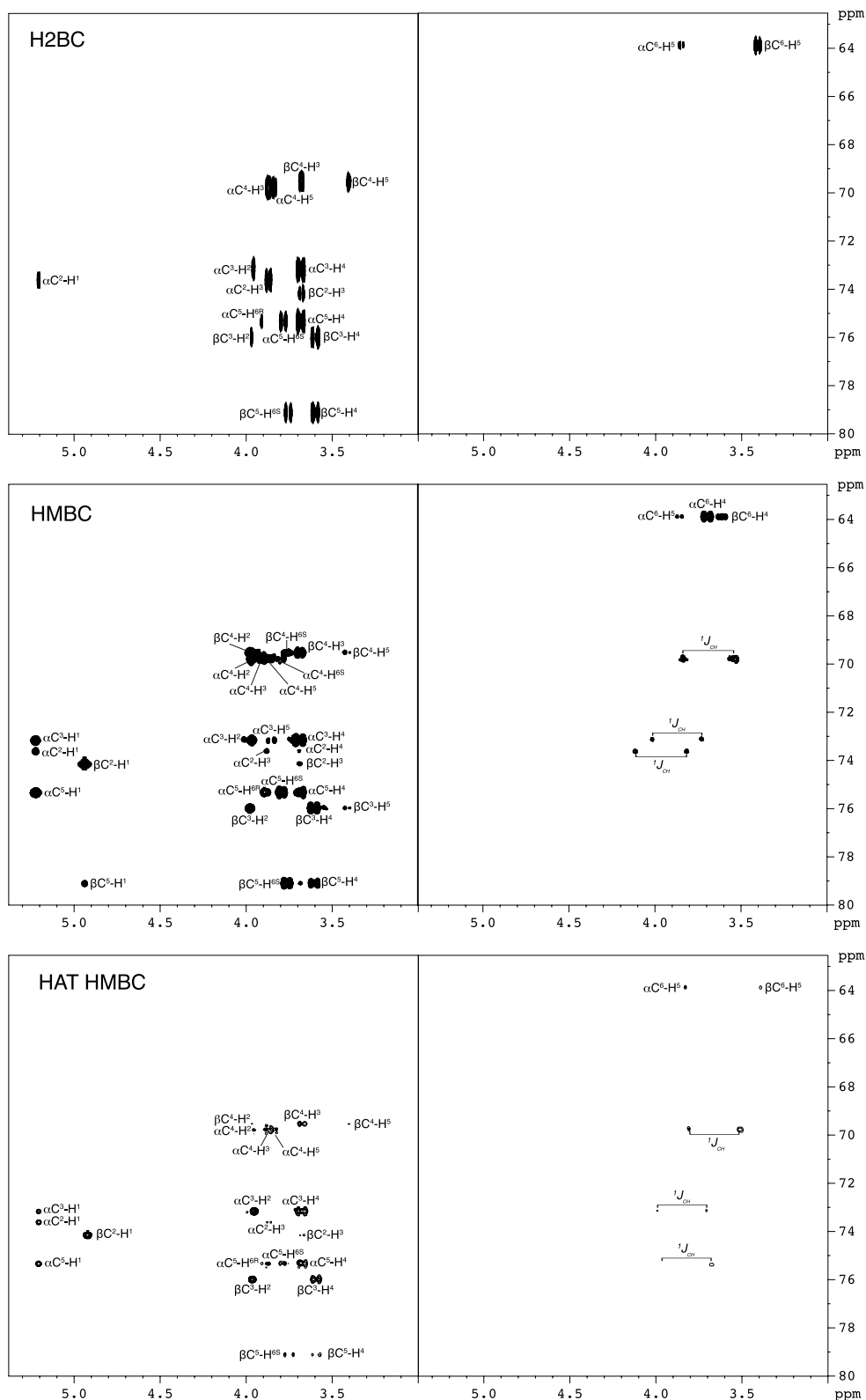


Fig. 2. Excerpts from multiplicity-edited spectra of 50 mM mannose in D_2O acquired on a Varian Unity Inova 500 spectrometer. The CH + CH_3 and C + CH_2 subspectra are shown in the left and right columns, respectively. The total number of transients was kept constant for each experiment, resulting in a total of 64 scans for 64 t_1 increments for edited H2BC and 16 scans for 256 t_1 increments for edited HMBC and edited HAT HMBC, the relaxation delay was 1 s, whilst the range for the third-order low-pass J filter was $140 \text{ Hz} < {}^1J_{CH} < 175 \text{ Hz}$. The number of complex points in the acquisition dimension was for all the experiments 1024 and the acquisition time 170.7 ms. The ${}^1J_{CH}$ used for the editing delay τ was 145 Hz. The data matrices covering 6 kHz and 3 kHz in F_1 and F_2 , respectively, were subjected to linear prediction in t_1 doubling the number of points and apodized using squared cosine in t_1 and a $\pi/4$ -shifted squared sine in t_2 prior to 2D Fourier transformation. (Top) Edited H2BC spectrum ($T = 21 \text{ ms}$), (middle) edited HMBC spectrum ($\Delta = 65 \text{ ms}$), (bottom) edited HAT HMBC spectrum ($\Delta = 65 \text{ ms}$). Signals arising from incompletely suppressed one-bond correlations are marked with ${}^1J_{CH}$.

respective carbons. Finally, editing can also help resolve overlapping peaks of different multiplicities [3,6].

As “hatting” is governed by $^{n+1}J_{\text{HH}}$ coupling constants between protons attached to a ^{13}C and another remote proton coupled to the ^{13}C , “hatting” becomes more efficient the more protons are attached to the ^{13}C , i.e. CH , CH_2 , and CH_3 in that order. For example, in a $^{13}\text{CH}_2$ group both couplings between a remote proton and the methylene protons cause antiphase character across the spectrum of the remote proton. Similar to other HMBC-type experiments, HAT HMBC can be performed with a single Δ delay or in a broadband version [8,9] with coaddition of spectra recorded with different Δ delays to achieve a more uniform excitation over a range of long-range J_{CH} coupling constants. The editing accuracy and the $^nJ_{\text{CH}}$ broadband feature are independent of each other.

There are further special cases, as e.g. for a CH_2 group where both, one, or none of the two protons can be associated with “hatting”. Single “hatting” can lead to J cross talk into the odd subspectrum whereas “double hatting” and “double no hatting” behave identically with respect to the even/odd editing and show up in the even subspec-

trum. “Double no hatting” contributions to the even subspectrum is for CH_2 groups at the ESCORT [10] level, i.e. compensated to first order in the deviation, $\Delta(^1J)$, of an actual 1J coupling constant and the value 1J_0 used in setting the $\tau/2 = (^1J_0)^{-1}/4$ delays. In practical applications, correct interpretation of HAT HMBC spectra hinges on observation of relative peak intensities compared to regular or edited HMBC spectra along with the two-bond correlations identified in the H2BC spectrum (vide infra).

In Fig. 2 are shown edited H2BC, HMBC, and HAT HMBC spectra of mannose. Mannose is an example where vanishing $^3J_{\text{HH}}$ coupling constants cause H2BC to fail in identifying all two-bond correlations, and hence call for such identification on the basis of a HAT HMBC spectrum. $^3J(\text{H1-H2})$ is small both in the α and β configuration [11], and as a result only a weak $\alpha\text{C2-H1}$ correlation peak out of the four possible ones (i.e. C2-H1 and C1-H2 (not shown) in both the α and the β configuration) is observed in the H2BC spectrum in Fig. 2. This is in contrast to the edited HMBC spectrum where all four are present, but it is not a priori obvious which peaks represent two-bond and which represent three-bond correlations. The edited

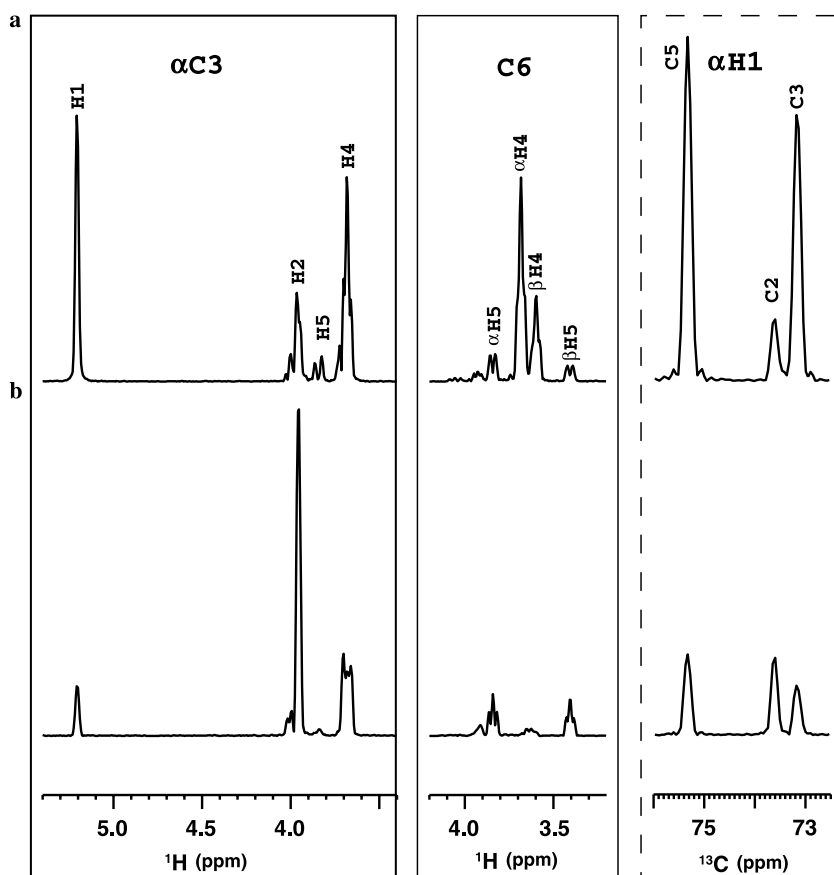


Fig. 3. 1D sections taken from edited (a) HMBC and (b) HAT HMBC spectra. The left-hand box shows sections taken along F_2 at the frequency of αC3 . As can be seen, the three-bond correlations to H1 and H5 are strongly attenuated in the HAT HMBC spectrum whilst the two-bond connectivity to H2 is enhanced. The middle box displays sections taken along F_2 at the frequency of C6. Here, the three-bond correlation to H4 in both anomers shows virtually complete suppression whilst the two-bond correlations to the H5 protons are slightly enhanced. The right-hand box shows equivalent sections to the first two examples, except that this time they are taken along F_1 . Here can again be seen the attenuation of the longer-range correlations whilst the two-bond correlation is slightly enhanced.

HAT HMBC spectrum exhibits several interesting features that are illustrated by 1D sections out of these spectra shown in Figs. 3 and 4.

Although no interpretations of this type of spectra can be absolute, comparison between the HMBC sections in Fig. 3a and the HAT HMBC sections in Fig. 3b indicates that the $\alpha\text{C3-H1}$, $\alpha\text{C3-H5}$, $\alpha\text{C6-H4}$, $\beta\text{C6-H4}$, and $\alpha\text{C5-H1}$ correlations are not over two bonds due to relatively strong attenuation in the HAT HMBC spectrum relative to the regular HMBC spectrum. Interestingly enough, the $\alpha\text{C3-H2}$, $\alpha\text{C6-H5}$ and $\beta\text{C6-H5}$, and $\alpha\text{C2-H1}$ two-bond correlations experience enhancement in the HAT HMBC spectrum relative to the regular HMBC spectrum.

As mentioned earlier, “hatting” changes relative phases of the components within multiplets and this can result in different multiplet patterns in HMBC and HAT HMBC spectra. Critical in this context is whether the multiplet components in the center have the same or opposite phases leading to enhancement or intensity reduction in the center, respectively. We call this the center effect and examples favoring HAT HMBC are $\alpha\text{C6-H5}$ and $\beta\text{C6-H5}$ with triplet structure compared to doublet structure in regular HMBC. The $\alpha\text{C3-H2}$ peak is an example of unusually

large enhancement in HAT HMBC. Examples of the center effect disfavoring HAT HMBC are the peaks $\alpha\text{C3-H4}$ in Fig. 3 and $\beta\text{C5-H4}$ and $\beta\text{C5-H6}^{\text{S}}$ in Fig. 4.

As a rule of thumb, peaks that are enhanced or “not attenuated too much” in the HAT HMBC spectrum relative to the regular HMBC spectrum are likely to represent two-bond correlations. Of course, there is no strict definition of “not attenuated too much” but cases with an obvious disfavoring center effect should definitely be included in this rule of thumb.

Two peaks in Fig. 4 deserve special comments. $\beta\text{C2-H1}$ is weak in the H2BC spectrum due to a very small $J(\text{H1-H2})$ coupling constant, and cannot a priori be interpreted as a two-bond correlation based on this spectrum because the intensity is at the same level as three-bond correlation peaks in the H2BC spectrum. It is an example where HAT HMBC is more sensitive than H2BC. The $\beta\text{C5-H2}$ peak is so weak in the H2BC spectrum that it in analogy to the $\beta\text{C2-H1}$ peak cannot be assigned as a two-bond correlation, but interestingly it is more intense in the HAT HMBC than in the regular HMBC spectrum indicating that it could be a two-bond correlation. However, because this correlation is relative-

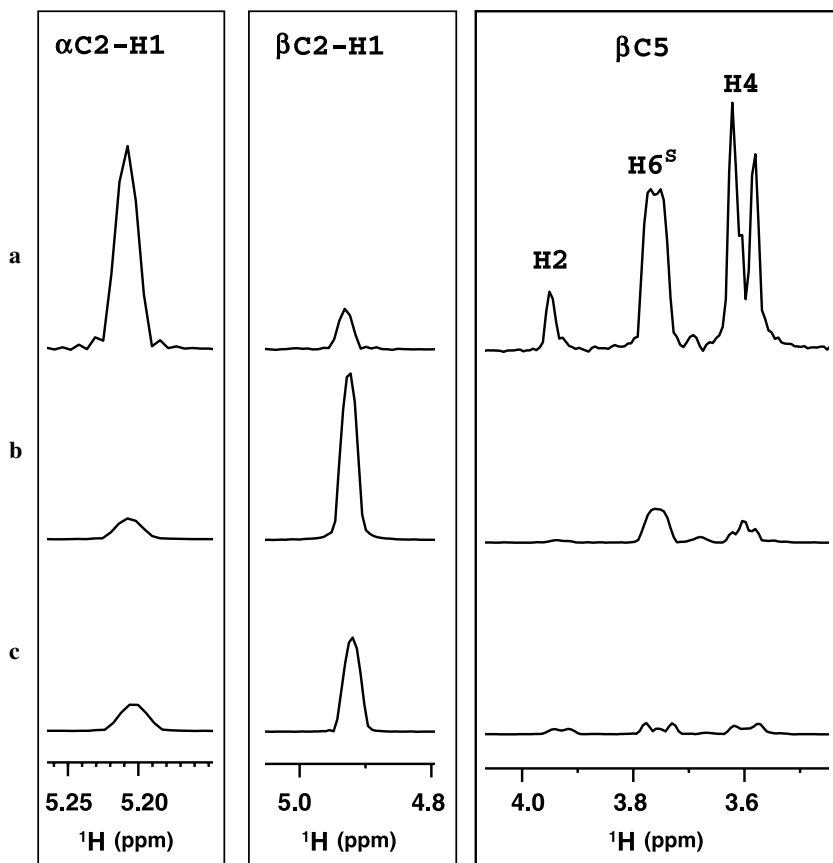


Fig. 4. 1D sections along F_2 taken from edited (a) H2BC, (b) HMBC, and (c) HAT HMBC spectra. To facilitate the comparison of the spectra, the H2BC spectrum is shown in the magnitude mode. The left-hand box shows sections taken at the frequency of αC2 and shows the correlation to αH1 . The middle box is equivalent to the left-hand box with the exception that it shows the equivalent correlation between βC2 and βH1 . Finally, the right-hand box shows sections taken at the frequency of βC5 .

ly weak in all three spectra it cannot be reliably assigned as to the number of bonds between the two spins and should be left unassigned until all obvious assignments have been made.

Obviously, individual intensities and phases of multiplet components within peaks reflect the pertinent coupling constants and chemical shifts, but such detailed knowledge of the spin systems is practically never available for molecules where HAT HMBC is relevant to perform. Therefore, interpretation of HAT HMBC spectra should be kept at the qualitative level and with the cautions indicated above. In regular HMBC spectra, the relative phases of multiplet components is a mess due to evolution under HH coupling constants during the pulse sequence, and cancellations within multiplets leading to lowered overall intensity is common. Hence quantitative interpretation of peak intensities in HMBC is not generally possible. HAT HMBC spectra are no different in this respect, the phases within multiplets are still messy, it is just a different mess because of the additional π phase difference across the J_{HH} associated with hatting. However, finer multiplet details or exact peak intensities are in routine applications neither relevant in HMBC nor in HAT HMBC spectra. Basically, the coherence transfer function is $\sin(\pi^n J_{CH} \Delta)$ in both HMBC and HAT HMBC, but this is not a good measure for the peak intensities because they are modified in an “unpredictable” way by evolution under usually unknown HH couplings and in HAT HMBC spectra by the additional π phase difference across the pertinent J_{HH} .

In conclusion, we have introduced HAT HMBC to help identify two-bond correlations that cannot be assigned as such in H2BC spectra due to low intensity caused by very small $^3J_{HH}$ coupling constants. HAT HMBC is an experiment to be performed after H2BC and regular HMBC to resolve possible open ends left by the spectra from these two experiments.

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