ATLANTIC REGION MAGNETIC RESONANCE CENTRE

Even More Structural Information from NMR – Introducing *H2BC*

Dr. Mike Lumsden
Coordinator, ARMRC
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Preliminary attempts at structural characterization of small to mid-sized molecules using NMR spectroscopy is normally done using a ^{1}H observe 1D experiment in some combination with basic 2D sequences such as COSY, HMQC/HSQC, and HMBC. Many of you still are directly observing the ^{13}C nucleus and in my last bulletin I introduced the availability of DEPTQ for this purpose. If ambiguities still remain after this preliminary stage, you are often forced to run additional experiments such as MQF-COSY, TOCSY, NOESY, HMQC-TOCSY, etc. Sometimes these ambiguities arise due to a couple of shortcomings associated with HMBC. The first is that the "*MB*" in HMBC stands for multiple bond but how does one get more specific and determine the number of bonds responsible for the correlation? The second issue is related to the generation of the multiple quantum coherence in HMBC and the complex dependence of the multiple bond J's on structure. ^{2}J CH values can be negative or positive and sometimes zero! If the J is zero, you can pulse from now into the next century and will not get an HMBC correlation! Furthermore, ^{3}J CH values show a type of a Karplus dependence on the C-C/C-H dihedral angle and so values around 90º are problematic. In practice, what these issues mean are that often HMBC is run more than once using different evolution delays to probe different values of the long-range couplings. Furthermore, one must always keep in mind that the lack of an HMBC correlation is NOT sufficient evidence on it's own that your proposed structure is incorrect.

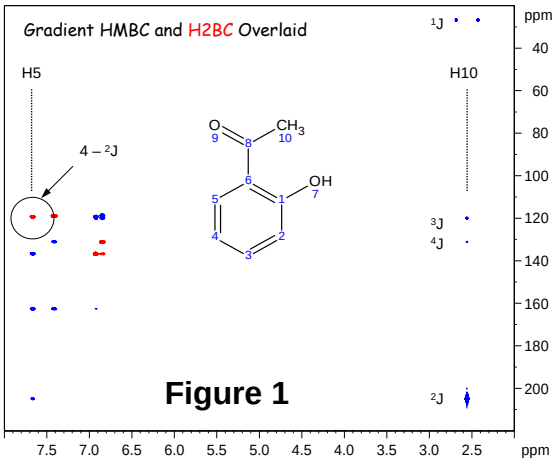

Figure 1

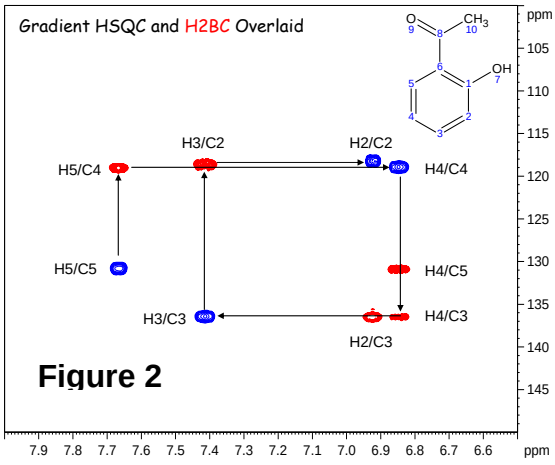

Figure 2

A recent paper out of Ole Sørensen's lab (*J. Am. Chem. Soc.* **2005**, *127*, 6154) introduced an experiment designed to help remove the aforementioned ambiguities associated with HMBC. They call the experiment H2BC as it is designed to show H-C correlations predominantly through 2 bonds only. Unlike HMBC, H2BC signals are generated by making use of both ^{3}J HH and ^{1}J CH couplings, removing all dependence upon ^{n}J CH. What this means in essence is that H2BC signals arise only from protonated carbon spins in which the protons have a vicinal homonuclear coupling partner. Experimentally, it has several advantages over HMBC, including pure absorption lineshapes, no broadening in the F1 dimension from homo and heteronuclear coupling due to a constant-time implementation, and the ability to apply broadband decoupling during acquisition. An additional advantage over HMBC, particularly important for heavier molecules, is that the overall sequence is shorter, attenuating the effect of signal losses due to transverse relaxation.

Figure 1 shows an overlay of both the 50 ms evolution (10 Hz) HMBC (blue contours) and H2BC (red contours) obtained at 500 MHz for the simple molecule o-hydroxyacetophenone. Concentrating first on the methyl group proton correlations, one sees 4 blue HMBC signals, one each for ^{1}J, ^{2}J, ^{3}J and ^{4}J! Although all the signals in theory are multiplets since no ^{13}C decoupling is performed, the 1 bond signal is an obvious doublet and arises due to the inability of the low pass filter in HMBC to remove this 1-bond correlation. Notice that H2BC signals are absent for this methyl group as the methyl protons due not have a vicinal proton coupling partner. For the aromatic proton H5, one sees 3 blue HMBC correlations and as it turns out all 3 are 3 bond signals (carbons 1, 3 and 8) – surprisingly both the potential 2 bond correlations to 4 and 6 are absent! However, the H2BC spectrum shows a signal due to the 2 bond correlation with carbon 4 (circled). Since carbon 6 is quaternary, it does not yield an H2BC correlation with H5.

Another advantage of H2BC comes when it is run in tandem with an HMQC/HSQC experiment. Overlaying these two spectra allows one to trace out INADEQUATE type connectivity information, although one must keep in mind that the "molecular walk" will come to a screeching halt at a HMQC/HSQC correlation where the next carbon along the path is quaternary. This is illustrated in Figure 2 for the protonated ring carbons in o-hydroxyacetophenone, where the walk starts at the H5/C5 HSQC correlation and ends at H2/C2.

In closing, I wish to stress that H2BC is in no way designed to replace HMBC but rather to supplement the results from this experiment, thereby hopefully simplifying your structure elucidation problems. T**wo words of caution are also in order -** *(a)* there is no 100% guarantee with H2BC that a given signal is through 2 bonds, although such a spurious signal will normally be weak. This could occur in systems with a non-vanishing ^{4}J HH coupling for example. *(b)* It is possible that a 2 bond signal could be missing in H2BC, which would infer that the relevant vicinal HH coupling is ~ zero, as would be the case when the H-C/C-H dihedral angle is ~ 90º.