Very Long-Range Correlations ($^{n}J_{C,H}$ n > 3) in HMBC Spectra

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The structural elucidation of natural products and complex organic molecules relies heavily on the application of proton detected heteronuclear NMR. Among these techniques, the HMBC NMR experiment remains as the most popular among the methods that sample long range coupling constants. The HMBC (C-H) experiment allows the assignment of structural fragments through correlations between protons and carbons separated by more than one bond, usually two or three ($^{2}J_{C,H}$ and $^{3}J_{C,H}$). It is also possible to obtain valuable information, sometimes crucial, through very long-range, or nonstandard correlations, $^{n}J_{C,H}$ n>3; they can, surprisingly, appear in standard HMBC spectra, or looked for by performing several HMBC experiments with different long-range delays and using a deeper threshold in the contour plot.

Key Words: Long-range HMBC, structural elucidation, natural products.

An analysis of articles published in recent years in the area of structural elucidation of organic compounds shows that, although valuable methods such ACCORD-MBC impeach-MBC and CIGAR-HMBC have been reported, the Heteronuclear Multiple Bond Correlation NMR experiment (HMBC) [1,2] remains as the most popular of all the methods that sample long range coupling constants. HMBC (C,H) provides a wealth of structural information through long-range correlation signals (or cross peaks) for ^{13}C, ^{1}H spin pairs that can span quaternary carbons or heteronuclei providing a way to connect molecular fragments into a complete structure [3-5]. Without a doubt, the use of HMBC in concert with either Heteronuclear Multiple Quantum Coherence (HMQC) [6] or Heteronuclear Single Quantum Correlation (HSQC) [7] has been extremely useful for the total structure elucidation and NMR spectral assignments of complex organic molecules.

However, in spite of the recognized high potential of HMBC, examination of NMR web pages, books and articles published in the last six years that are related to this subject, shows that the belief remains that HMBC, in its standard form, is useful mainly to observe correlations across either two- or three-bond, and the observation of couplings at four-bond would be very exceptional, arising mainly when the method is tuned to observe these couplings. However, the early observation of a crucial four-bond C,H correlation in the HMBC spectrum of the antibiotic distamycin A [8] (Figure 1) and the several examples of this kind of correlation that we have reported [9] refute this belief.

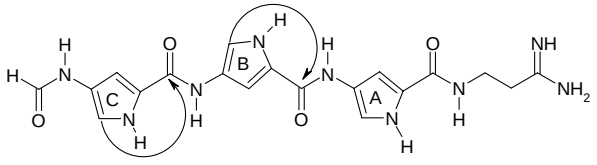

Figure 1: Four-bond correlations observed for distamycin A.

In our opinion, the persistence of this belief is a consequence of a kind of self-fulfilling prophecy. If the HMBC method is learned in the form previously mentioned, then when a very long range correlation

($^{n}J_{C,H}$ n>3) appears, it will probably be discarded, unless the information that it provides is absolutely necessary.

With regard to the observation of $^{n}J_{C,H}$ n>3, two different opinions can be argued: one of them emphasizes the disadvantages associated with an increased number of H,C cross peaks along with the uncertainty of the number of bonds involved with these C,H couplings. As consequence of this, the valuable information that might be obtained through the use of heteronuclear long-range correlations across four or more bond ($^{n}J_{C,H}$ n>3) generally is discarded. The other opinion, despite the lack of discrimination, is focussed on the great quantity of structural information that can be obtained with a bigger number of long-range correlation peaks that can be translated to the bonding network. This opinion is the basis of the further development of pulse sequences after HMBC, such as D-HMBC [10], 3D-HMBC [11], CT-HMBC [12], ACCORD-HMBC [13], IMPEACH-MBC [14] and CIGAR-HMBC [15]. These methods are able to sample a great number of long-range coupling constants.

Six years have elapsed since our previous review on this subject, where we showed many examples of long-range correlations across four and five-bond in a diversity of chemical structures. More recently, we have reported that not only complex organic molecules can exhibit this kind of very long-range or non-standard correlations, but simple compounds also display these features [9].

One of the frontiers in structural analysis of organic molecules is the development of expert systems allowing computer-assisted structure elucidation based on spectral data. The impressive successes of one of them, the Structure Elucidator system (StrucEluc) [16] is due, in the opinion of the authors, to its capabilities for elucidating molecular structures from 2D NMR spectroscopic data. It must be recognized that this program was developed to solve problems where 2D NMR data were assumed to contain an unknown number of very long range correlations. These correlations, obtained from COSY or long-range heteronuclear shift-correlation spectra corresponding to four or more bonds ($^{n}J_{H,H}$, and $^{n}J_{H,C}$, n>3 correlations), were named as nonstandard by the authors [16,17]. The achievements and developments of 2D NMR spectroscopy, which are now both routine and widespread, provide high quality spectra under standard conditions, which allow the analysis of even very tiny, although informative, cross peaks. Today the advantages offered by the incorporation of pulsed field gradients (PFG) into high-resolution NMR pulse sequences, combined with advanced software tools available to acquire and process multidimensional NMR experiments, has allowed even the non-experienced user to record and process the HMBC sequence with PFG [18]. Moreover, the advent of cryogenic probes with their improved sensitivity could help to observe this kind of correlations more frequently.

The tuning of the HMBC experiment is achieved by setting the Δ_2 preparation period, the so called long-range delay, to a sufficiently long time to allow the small long-range proton-carbon couplings to evolve and produce the antiphase displacement of vectors required for the subsequent generation of heteronuclear multiple quantum coherence, and is calculated from $\Delta_2 = 1 / (2\ ^{n}J_{C,H})$ [5]. Since molecules have a range of $^{n}J_{C,H}$ values, typically from 2 to 15 Hz [4,5], Δ_2 should be at least 100 ms. In practice, a delay shorter than the theoretical value is employed, in order to avoid the decay of the ^{1}H magnetization during this delay, particularly for large molecules.

For routine applications, Δ_2 is usually set from 60 to 80 ms [3-5]. This makes the cross-peaks arising from values of $^{n}J_{CH}$ that are well removed from the average value to be significantly attenuated.

Since small molecules tend to have slower relaxation rates, longer Δ_2 delays can be used successfully in the search for more connectivities through smaller couplings. A maximum of 200 ms has been recommended to detect cross-peaks arising from four-bond correlations, which are described as most likely to occur when the coupling pathway contains either unsaturation or when it has the planar zig-zag (W coupling) configuration [4], as commonly observed in long-range proton-proton coupling. However, these generalizations might lead to confusion. For example, there has been reported the use of Δ_2 values from 50 to 400 ms to record HMBC spectra of phenolic compounds [19]. Also, the observation of five-bond correlations by using a standard value of Δ_2 (65 or 80 ms) in HMBC spectra has been reported [20-22]. Similarly, several examples of $^{4}J_{CH}$ have been observed in a great variety of molecules, and coupling pathways [9].

The choice of Δ_2 is generally made on an arbitrary basis rather than from knowledge of the actual value of the couplings. On this basis, a first approximation to observe a greater number of long-range correlations is to perform several HMBC experiments with different long-range delays combined with a deeper threshold in the contour plot. In this way, new correlations could be observed and some of those initially seen may disappear. Alternatively, some of the new pulse sequences focused on the question of optimisation of long-range delay, mentioned previously, of which CIGAR-HMBC has been described as the best [5].

A series of examples where the observation of long-range ^{1}H-^{13}C correlations, described using the HMBC experiment, were employed to elucidate the structures of a variety of compounds, in several deuteriated solvents, are presented. In most cases the authors do not refer explicitly to the long range correlation delay used. Therefore, we will only mention it when the original authors have pointed it out.

The next Figures show only correlations from four- and five-bond; two and three bond correlations are not shown. The arrows go from proton to carbon.

Correlations across five bonds

The observation of a $^5J_{C,H}$ correlation for 4,4-dimethylanthracen-1,9,10(4*H*)-trione (Figure 2) and some derivatives substituted in the aromatic ring was essential for the total assignment of the ^{13}C spectra of these molecules. The observation of $^5J_{C,H}$ and $^4J_{C,H}$ in this series depends on the pattern and nature of the substituents [20,21]. The HMBC experiments were performed at 300 MHz (^{1}H) and 75.47 MHz (^{13}C), in $CDCl_3$, with Δ_2 = 65ms. The modification of Δ_2 from 65 ms ($^nJ_{C,H}$ = 7.7 Hz) to 100 ms ($^nJ_{C,H}$ = 5 Hz) was not sufficient to show any differences in the observed correlations (Figure 2a).

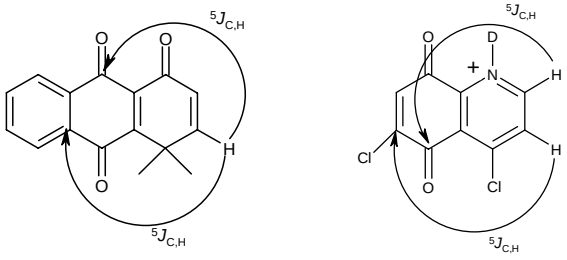

Figure 2: (a) five-bond correlations observed for 4,4-dimethylanthracen-1,9,10(4*H*)-trione with Δ_2 = 65 ms; (b) Five-bond correlations observed for 4,6-dichloroquinoline-5,8-dione with Δ_2 = 80 ms.

Correlations across five-bond have been observed also for heterocyclic quinones [22]. In an HMBC experiment carried out in CF_3CO_2D at 500.13 and 125.78 MHz for ^{1}H and ^{13}C, respectively, with Δ_2 = 80 ms, several $^4J_{C,H}$ and two $^5J_{C,H}$ correlations were observed (Figure 2b).

The HMBC of ethyl chrotonate, a structurally simple compound, also shows a five-bond correlation in its HMBC spectrum, in $CDCl_3$ solution, (Δ_2 = 65ms), through a coupling path with a high degree of conformational freedom [22] (Figure 3a).

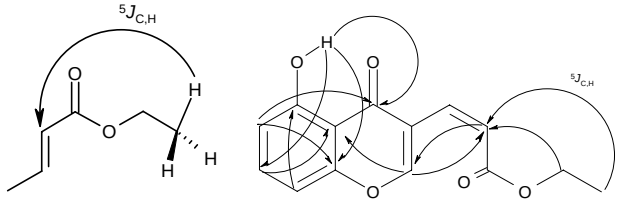

Figure 3: (a) Four and five-bond correlations observed for ethyl chrotonate with Δ_2 = 65 ms; (b) Four and five-bond correlations for chromone derivative with Δ_2 = 130 ms.

With the same Δ_2, the HMBC spectra of (*E*) and (*Z*) 3-(4-oxo-4*H*-chromen-3-yl)-acrylic acid ethyl esters, compounds bearing the acrylate moiety, do not show these long-range correlations, although some $^4J_{C,H}$ correlations were observed. In a study to obtain a criterion that allows a more appropriate choice of Δ_2 in this type of molecular system, several HMBC experiments, in $CDCl_3$ solution, were conducted with increasing Δ_2 long-range delays. It was observed that a slight increment of Δ_2, up to 130 ms, allowed the observation of the $^5J_{C,H}$ correlation between the methyl protons and C_α to the carbonyl group, previously observed for ethyl chrotonate. Several $^4J_{C,H}$ correlations through no W coupling paths were also observed in these systems (Figure 3b). From this study, an additional HMBC spectrum using a long Δ_2 delay (200 – 300 ms) was recommended in order to obtain valuable structural information through long range correlations [23]. A similar long-range correlation across a methyl acetate group has been reported for the terpene dichapetalin [9].

Long-range correlations across four bonds

The ^{13}C shift assignments of the sulfamate indoles, ancorinolates A and B, isolated from the sponge *Ancorina* sp., were made through HMBC long-range correlations [24]. The unambiguous assignment of C-5 and C-6 is a difficult task. This is because H-7 and the proton of the hydroxyl group in both

compounds, and H-4 in ancorinolate B, show HMBC correlations with C-5 and C-6, making the signals belonging to both carbons indistinguishable. However, the HMBC spectrum (500 MHz) of the acetylated derivative of ancorinolate B shows a crucial four-bond correlation across the ester group, between the acetate methyl protons and C-5. Since acetylation in aromatic compounds causes shielding at the ipso carbon and deshielding at the carbons in the *ortho* position, the signals could be assigned, (Figure 4).

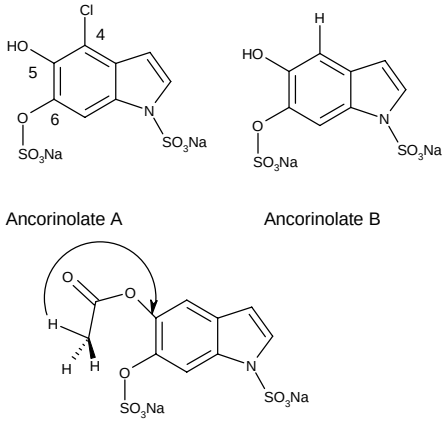

Figure 4: Four-bond correlations observed for acetylated ancorinolate B.

Several four-bond correlations are observed in the HMBC spectrum of the synthetic heterocyclic quinone 6-chloro-9-azaanthra[2,3-b]thiazole-5,10-dione (500 MHz), using CF_3CO_2D as solvent [22] (Figure 5a). Avicennone A, isolated from the twigs of *Avicennia marina*, shows a four-bondcorrelation in its HMBC spectrum (300 MHz), using $CDCl_3$ as solvent [25] (Figure 5b).

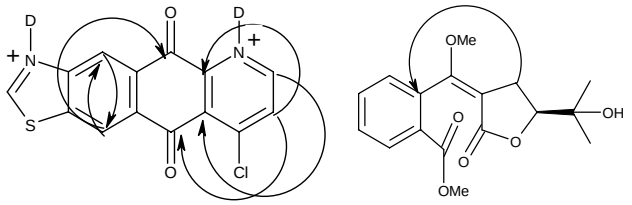

Figure 5: (a) Four-bond correlations observed for 9-chloro-6-azaanthra(2,3-b)tiazol-5,10-dione with Δ_2 = 80 ms; (b) Four-bond correlations observed for avicennone A.

The carbon skeleton of 1,2-dihydro- or 1,2,3,3'-tetrahydro-2,3-didehydrocaulerpenyne, present in sixteen acetylene sequiterpenoid esters isolated from the green alga *Caulerpa prolifera,* show two $^4J_{C,H}$ correlations in their HMBC spectra (400 MHz), using $CDCl_3$ as solvent [26] (Figure 6).

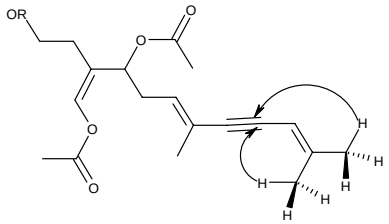

Figure 6: Four-bond correlations observed for 1-caulerpenyne derivatives.

The polyepoxysqualene-derived triterpenes, yardenones A, B and C, isolated from the marine sponge *Axinella* cf. *bidderi*, exhibit four-bond correlations in their HMBC spectra in $CDCl_3$ solution (500 MHz) [27] (Figure 7).

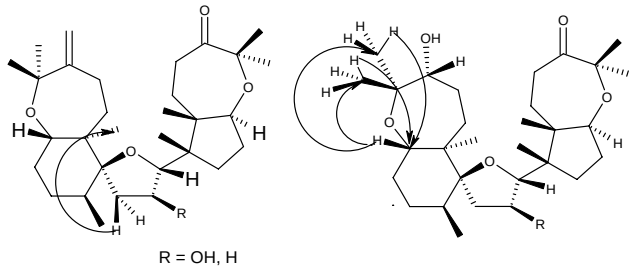

Figure 7: Four-bond correlations observed for yardenones A-C.

The structure of an anti-inflammatory thiazine alkaloid, ascidiathiazone B, isolated from the ascidian *Aplidium* sp. was elucidated by comparison of the crucial four-bond correlations between the methylene neighbor of the sulfur atom and a carbonyl quinonic carbon across the sulfone group in both ascidiathiazone B and a synthetic regioisomeric analogue [28] (Figure 8).

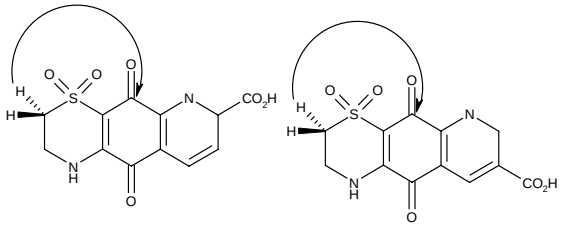

Figure 8: Four-bond correlations observed for ascidiathiazone B.

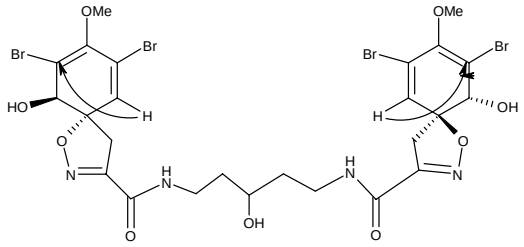

Figure 9: Four-bond correlations observed for (+)-12-hydroxyhomoaerothionin.

(+)-12-Hydroxyhomoaerothionin, a tetrabromospiro cyclohexadienylisoxazole isolated from the marine crinoid *Himerometra magnipinna,* exhibits a four-bond correlation across a dienyl moiety. The HMBC spectrum was recorded at 500 MHz in acetone-d_6 solution [29] (Figure 9).

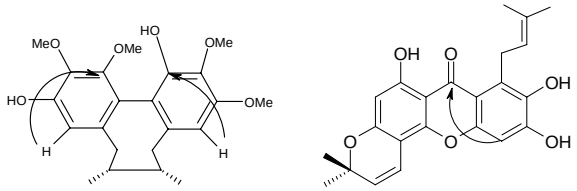

Figure 10: (a) Four-bond correlations observed for rubisandrin A; (b) Four-bond correlations observed for termicalcicolanone B.

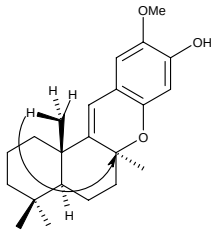

Figure 11: Four-bond correlations observed for puupehenone.

A similar kind of coupling was observed in the HMBC spectrum of the lignan rubisandrin A, isolated from the fruits of *Schisandra rubriflora* (400 MHz in $CDCl_3$) (Figure 10a). The cytotoxic xanthone termicalcicolanone B, isolated from the Madagascan plant *Terminalia calcicola*, showed a four-bond correlation between an aromatic proton and a carbonyl group (500 MHz; DMSO-d_6) (Figure 10b). Puupehenone (Figure 11) has been repeatedly encountered in sponges from four distinct orders. This compound exhibited a four-bond correlation in the HMBC spectrum (500 MHz; $CDCl_3$ solution) [30].

The alkaloid sanguinone A and the indoloquinone sanguinolentaquinone, isolated from *Mycena sanguinolenta*, show the same kind of four-bond correlation in their HMBC spectra at 600 MHz in CD_3OD and 500 MHz in D_2O, respectively (Figure 12a). A four-bond correlation was used to determine the structure of hyrtiazepine, an azepino-indole-type alkaloid isolated from the Red Sea marine sponge *Hyrtios erectus*. (600 MHz; CD_3OD) (Figure 12b).

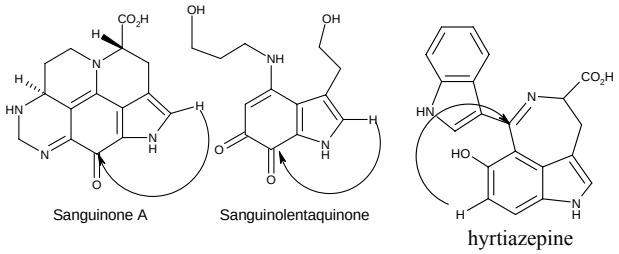

Figure 12: (a) Four-bond correlations for sanguinone A and sanguinolentaquinone; (b) Four-bond correlations for hyrtiazepine.

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