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3D-HMBC, A New NMR Technique Useful for Structural Studies of Complicated Molecules

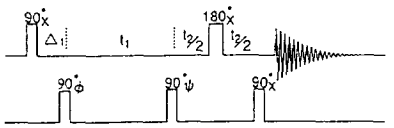
Kazuo Furihata and Haruo Seto*
Division of Agriculture and Agricultural Life Sciences,
*Institute of Molecular and Cellular Biosciences, University of Tokyo,
Yayoi, Bunkyo-ku, Tokyo 113, Japan

Abstract: A new NMR technique, 3D-HMBC, which is superior to conventional 2D-HMBC in its sensitivity for detection of long-range ^{1}H-^{13}C couplings is described.

For structural studies of complicated natural products by NMR spectroscopy, it is essential to detect ^{1}H-^{13}C long-range couplings. HMBC,[1] which enables detection of ^{1}H-^{13}C couplings with high sensitivity, is one of the most powerful NMR techniques suited for such an experimental purpose. In order to get good HMBC spectra, one of the experimental parameters, delay time ($\triangle$) must be set to a proper value by considering several parameters such as the magnitude of long-range ^{1}H-^{13}C coupling constants and splitting patterns of protons used for detecting ^{1}H-^{13}C cross peaks. These parameters, however, are variable depending on the relationships between a given proton and its long-range coupled carbons, and therefore, it is impossible to select an all-purpose delay time which will give satisfactory results with all ^{1}H-^{13}C long-range relations. As a compromise, the delay time value is generally set to 60 msec for ordinary HMBC experiments[1] resulting in the decrease of the sensitivity for detection of some ^{1}H-^{13}C long-range correlations.

The sensitivity of HMBC is also diminished by multiple couplings of a given proton with several adjacent protons, this being especially striking with methylene proton signals in a complicated proton spin system. This phenomenon is caused by fan-out of proton signals of concern due to coupling to many proton signals during the evolution period.

Fig. 1

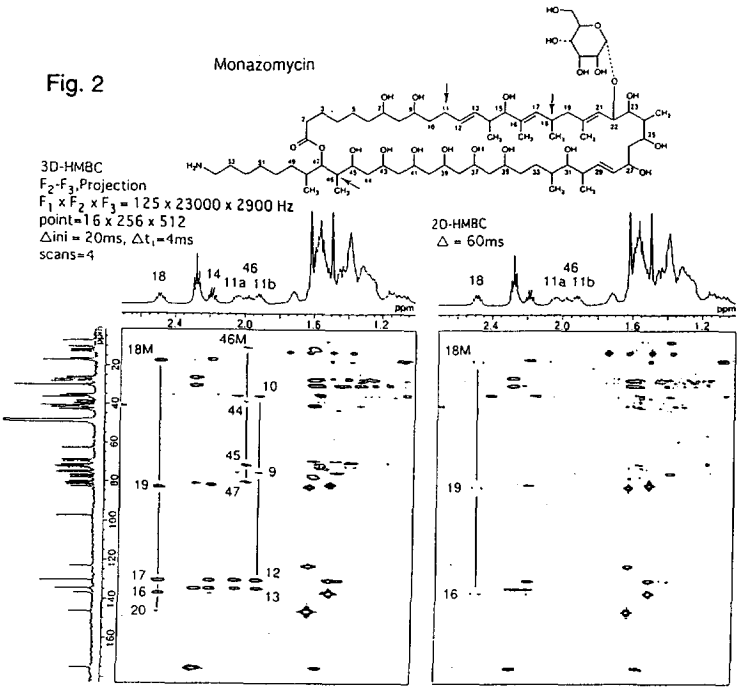


In order to overcome these problems, we have improved the conventional 2D-HMBC to develop a new NMR technique, 3D-HMBC. Its pulse sequence is shown in Fig. 1. The delay time $\triangle_2$ and t_1 in the 2D-HMBC have been replaced by t_1 and t_2, respectively, in the 3D-HMBC technique with $\triangle_1$ being set to 3.5 msec.

The great improvement for signal detection is exemplified in Fig. 2, which shows the 2D- and 3D-HMBC spectra of the antibiotic, monazomycin[2] (20 mg in 0.4 ml CD_3OD). The 3D experimental conditions were as follows; $F_1(^{1}H)$ X $F_2(^{13}C)$ X $F_3(^{1}H)$ =125 X 23000 X 2900 Hz, data points 16 X 256 X 512, the initial value of t_1 was 20 msec with the increment ($\triangle_1$) of 4 msec for 15 steps, measuring time was ca. 7 hrs with PFG. The 2D-HMBC spectrum was taken by employing the same experimental parameters as the 3D experiment except that 64 scans were used instead of 4 scans for the latter (total time ca. 7 hrs). In the 2D-HMBC spectrum (Fig. 2, right), only weak cross peaks are observed between a broad proton signal, H-18 and C-18M, C-19 and C-16, and no cross peaks are detected with a methine H-46 and a methylene H-11a and H-11b. The reason for failure to observe the cross peaks of these signals was apparently due to the improper setting of the delay time. In contrast, in the 3D-HMBC spectrum (Fig. 2, left) which covers the delay time region from 20 msec to 80 msec, strong cross peaks are evident, for example, from H-18 to the C-18M, C-19, C-17 and C-16 signals. These results clearly show that 3D-HMBC is quite a useful technique when the information on the connectivity around broad proton signals is not obtainable due to

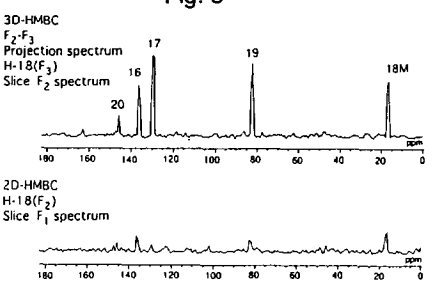
complex splitting patterns of the concerned signals and due to fast transverse relaxation of methylene signals.[3] The slice data shown in Fig. 3 emphasizes the dramatic increase of the signal intensities of the cross peaks between H-18 and relevant carbons. It should be noted that only 4 scans were used for taking each slice in the 3D experiment as opposed to 64 scans for the 2D experiment. Nevertheless, the 3D experiment which can cover the "best-hit" delay time for each ^{1}H-^{13}C long-range relation[4] gives far better results than the 2D experiment.

Fig. 2



From our experiences to date, 3D-HMBC is the method of choice for observing ^{1}H-^{13}C long-range couplings and it almost always gives better results than conventional HMBC. The requirements for carrying out the 3D-HMBC experiments, such as the large data storage area and 3D-data processing, are not an obstacle with the state-of-the-art NMR instruments.

Fig. 3



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2. H. Nakayama, K. Furihata, H. Seto and N. Otake: *Tetrahedron Lett.*, **22**, 5217-5220 (1981).
3. Due to the short initial delay time (20 msec) employed in 3D-HMBC, signals decaying during the delay time in 2D-HMBC (60 msec) can be detected more effectively with improved S/N in the 3D spectra.
4. If one wants to detect very small ^{1}H-^{13}C long-range couplings, the initial delay time must be set to a longer value.

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