CN−HMBC: A powerful NMR technique for the simultaneous detection of long−range H−1,C−13 and H−1,N−15 connectivities

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CN-HMBC: A Powerful NMR Technique for the Simultaneous Detection of Long-Range $^{1}H,^{13}C$ and $^{1}H,^{15}N$ Connectivities

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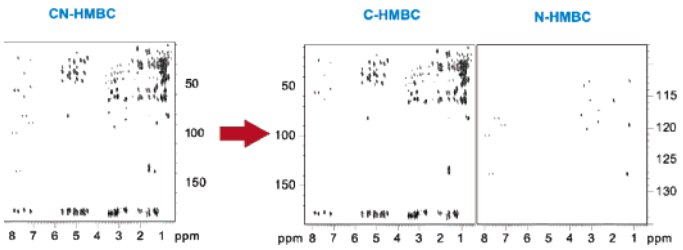
ABSTRACT



A new one-shot NMR experiment (CN-HMBC) is proposed for the simultaneous acquisition of 2D $^{1}H,^{13}C$ and $^{1}H,^{15}N$ HMBC spectra. Important sensitivity enhancements (up to 41% simultaneously for both ^{13}C and ^{15}N) or time savings (about 50%) can be achieved when compared to the separate acquisition of individual HMBC spectra. The experiment is highly recommended for the complete structural analysis and simultaneous chemical shift assignments of protonated and nonprotonated ^{13}C and ^{15}N resonances in nitrogen-containing organic compounds.

The HMBC (heteronuclear multiple-bond correlation) experiment[1] is an indispensable strategic NMR tool in the structural determination and chemical shift assignment of organic molecules. With the advent of pulsed-field gradients, clean $^{1}H-^{13}C$ HMBC (C-HMBC) spectra are now routinely obtained under fully automated acquisition setup conditions, and therefore, it can be considered the gold standard to trace out long-range heteronuclear connectivites to protonated and non-protonated ^{13}C nuclei.[2] The HMBC experiment also finds unlimited applicability for establishing heteronuclear correlations on a wide number of other different X heteronuclei as reported for ^{15}N, ^{19}F, ^{29}Sn, or ^{31}P. Particularly, ^{15}N NMR parameters are sensitive indicators of important structural and electronic arrangements and the use of $^{1}H-^{15}N$ HMBC (N-HMBC) spectra to get experimental evidence of the presence of protonated and nonprotonated nitrogen atoms in organic, bio-organic, and organometallic compounds is of particular interest.[3] Long-range N-HMBC data can be used to study and resolve a great number of chemical questions such as the full characterization of natural and synthetic products,[4] to differentiate regioisomers,[5] to determine the site of N-protonation,[6] N-oxidation,[7] nitration,[8] N-protection,[9] or N-substitution,[10] to analyze degradation products and metabolic pathways, to study tautomeric equilibria and hydrogen

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bonding,[11] or to probe metal-binding in organometallic chemistry.[12]

Because a concerted analysis of $^{1}H,^{13}C$ and $^{1}H,^{15}N$ HMBC spectra is usually required for establishing unambiguous heteronuclear correlations or for structure verification in nitrogen-containing compounds, an one-shot NMR experiment (termed CN−HMBC) (Figure 1) is proposed for the simultaneous acquisition of these two different C− and N−HMBC spectra under advantageous conditions.

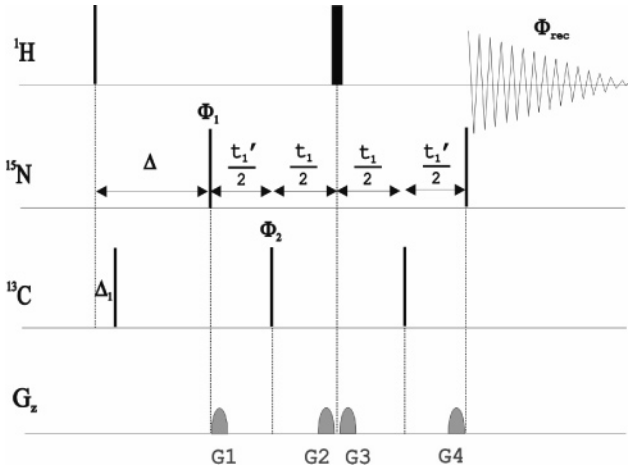

Figure 1. Basic pulse scheme of the CN-HMBC experiment suitable for the simultaneous acquisition of absolute-mode $^{1}H,^{13}C$ and $^{1}H,^{15}N$ 2D HMBC spectra. To discriminate correlations originated from N and C nuclei, two datasets are recorded with a minimum two-phase cycle: (A) $\phi_1 = \phi_2 = x,-x$ and (B) $\phi_1 = -x,x$ and $\phi_2 = x,-x$ with the receiver phase always set to $\phi_{rec} = (x,-x)$. After data acquisition, these two time-domain datasets are added/subtracted to afford separate C- and N-HMBC spectra after conventional data processing. More experimental details are provided in the Supporting Information.

This approach offers important sensitivity enhancements per time unit for both ^{13}C and ^{15}N or, in other words, reduced acquisition times when compared to the separate acquisition of individual C- and N-HMBC data. Furthermore, the new CN-HMBC pulse retains all features and simplicity related to the traditional HMBC experiment such as the use of pulsed-field gradients for coherence selection, a similar overall duration and pulse timing design, a minimum number of pulses, and easy setup protocols that yield clean C- and N-HMBC spectra in a very simple, automated way.[13] For example, CN-HMBC spectra were recorded on a natural product (the alkaloid strychnine, **1**), a cycloundecapeptide (cyclosporine, **2**), and an organometallic ruthenium(II) complex containing several nitrogen−multidentate ligands **3** (Figure 2).

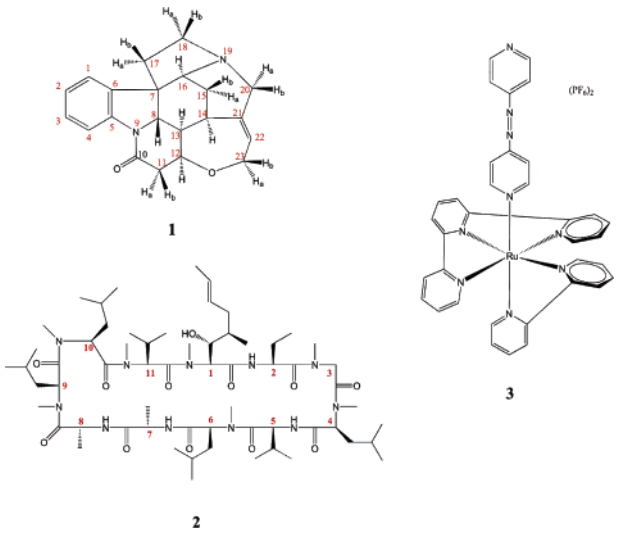

Figure 2. Test nitrogen-containing molecules used in this study

Similar signal-to-noise ratios are obtained when comparing the sensitivity of a single CN-HMBC spectrum (Figure 3B) acquired with the same number of scans and with the same experimental time as each individual C- and N-HMBC

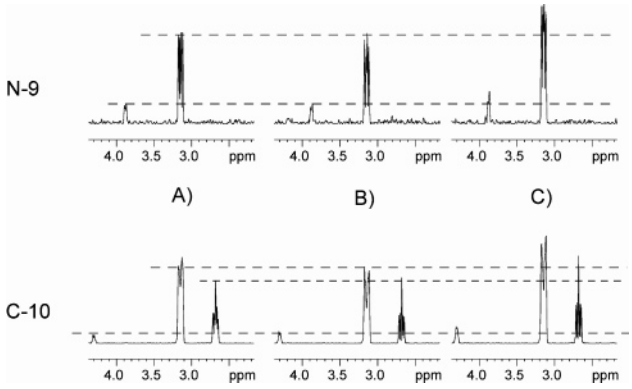

Figure 3. 1D row sections taken at C-10 (172 ppm) and N-9 (151 ppm) of **1**: (A) individual slices extracted from 2D C- and N-HMBC spectra acquired separately; (B) individual slices extracted from the 2D CN-HMBC spectrum acquired with the same number of scans as each individual HMBC experiment shown in A; (C) Individual slices taken from the 2D CN-HMBC spectra acquired with the same total measuring time as both spectra in A.

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spectra acquired separately with the standard sequences (Figure 3A). This demonstrates that the CN-HMBC pulse sequence does not introduce additional drawbacks, and it works similarly to single-nucleus HMBC experiments in terms of sensitivity, relaxation, and effectiveness. The main advantage of the CN-HMBC experiment relies in its better sensitivity levels achieved per time unit because two different spectra are obtained simultaneously. Thus, assuming that N scans are needed for an individual C-HMBC and another additional N scans for the N-HMBC, a total of 2N scans should be required to dedicate the same acquisition time for the simultaneous CN-HMBC experiment. In this way, a theoretical 41% signal-to-noise enhancement is expected to be reached. In practice, to separate the two types of responses found in the CN-HMBC experiment, two datasets are separately stored for each t_1 increment with phase inversion of the first 90° ^{15}N pulse (N scans with $\phi_1 = x$ (data A) and N scans for $\phi_1 = -x$ (data B). After linear combination (A ± B), separate N- and C-HMBC spectra are obtained with an important sensitivity enhancement (compare parts A and C of Figure 3). The resulting CN-HMBC spectra are best presented in absolute value, and phase or baseline corrections are therefore not required after Fourier transformation.

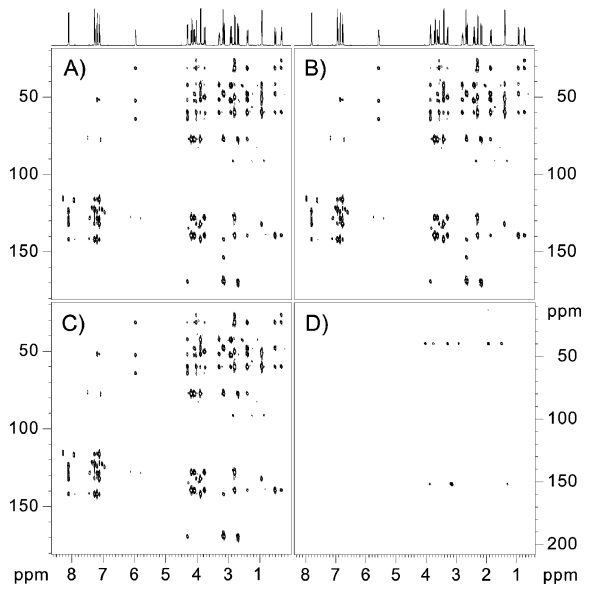

Figure 4. 2D CN-HMBC spectra of **1** showing the basic acquisition and processing steps followed in this work: (A, B) Two complementary datasets are acquired with relative phases $\phi_1 = x$ and $\phi_1 = -x$ respectively. Appropriate linear combinations (A ± B) followed by conventional processing provide the two separate (C) C-HMBC and (D) N-HMBC spectra with enhanced sensitivity as shown in Figure 3.

Figure 4A shows the corresponding CN-HMBC spectrum of **1** acquired with the same relative phases $\phi_1 = x$ and $\phi_2 = x$. Although all long-range correlations to ^{13}C and ^{15}N are displayed, it is not possible to distinguish directly between such C- and N-cross-peaks. The acquisition of a complementary CN-HMBC data with relative phases $\phi_1 = -x$ and $\phi_2 = x$ affords a relative phase inversion of the N cross-peaks, although they are not visible from the corresponding magnitude-mode representation (Figure 4B). Addition and subtraction of these two time-domain data followed by the conventional data processing afford the separate clean C-HMBC and N-HMBC spectra (Figure 4C,D). Clearly, two nitrogen resonances belonging of the N-19 amine (40 ppm) and the N-9 amide (152 ppm) functional groups and their correlation peaks can be easily distinguished and assigned (Figure 4D).

Most of the experimental settings required to setup the CN-HMBC experiment follow similar arguments to the traditional HMBC experiment.[14] The initial evolution Δ delay can be optimized to the same value for ^{13}C and ^{15}N (compromise values between 60−80 ms can be routinely used) because long-range J(CH) and J(NH) coupling constants present similar magnitudes which rarely exceed 10−15 Hz. A ^{13}C low-pass J filter is recommended to avoid accidental overlapping and ambiguous interpretation due to the numerous direct ^{1}H−^{13}C correlations due to ^1J(XH) in the resulting C-HMBC spectrum (Figure 5B). On the other hand, the presence of direct NH cross-peaks resonating as doublets with a large splitting of about 90 Hz between 7 and 8 ppm in the N-HMBC spectrum (Figure 5C) helps to distinguish them from nonprotonated nitrogens and avoids the acquisition of a separate one-bond NH correlation spectrum. Furthermore, valuable two-bond correlations are also obtained for the seven nonprotonated nitrogens belong-

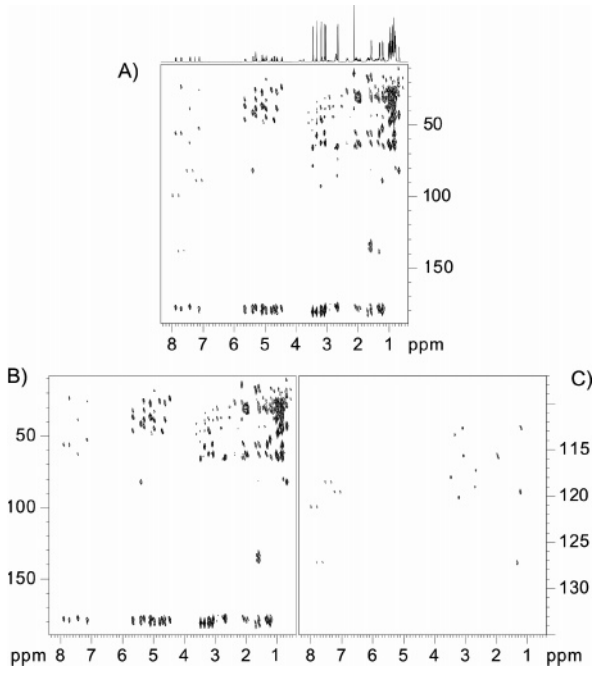

Figure 5. (A) Two-dimensional CN-HMBC spectrum of **2**. Separate ^{13}C-HMBC (B) and ^{15}N-HMBC (C) are obtained after proper time−domain data addition/subtraction, as described in Figure 4.

ing to *N*-methyl groups which provide a full ^{1}H, ^{13}C, and ^{15}N chemical shift assignment.

The CN-HMBC experiment can also find enormous interest to prove metal binding in organometallic chemistry. The diamagnetic [Ru(trpy)(bpy)(azpy)]$(PF_6)_2$ complex where the central ruthenium atom is coordinated to several piridynil-containing multidentate ligands has been chosen as an example (Figure 6). The simultaneous information extracted

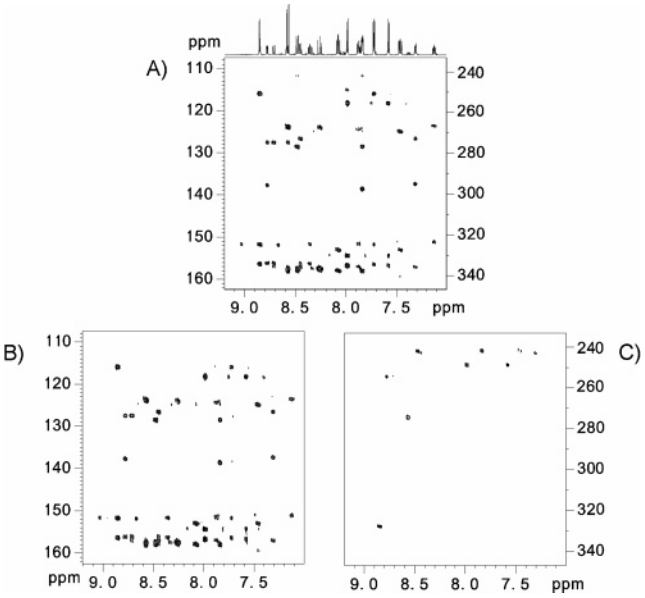

Figure 6. (A) Two-dimensional CN-HMBC spectrum of **3**. Separate ^{13}C-HMBC (B) and ^{15}N-HMBC (C) are obtained after proper time−domain data addition/subtraction, as described in Figure 4.

from both C-HMBC and N-HMBC data allows the complete chemical shift assignment of nine nonprotonated nitrogen atoms and six quaternary carbons. In particular, the knowledge of the ^{15}N chemical shift is a sensitive reporter of the local environment for a given nitrogen atom, and in this case, it is a clear experimental evidence of the presence of the N−Ru bond. This information allows us to distinguish between linked and nonlinked connectivities in multicoordinated N-ligands and also help us to confirm the presence of mononuclear or dinuclear species.[15] Five of the six pyridinyl nitrogens resonate in the 240−275 ppm region, whereas a non-Ru-linked nitrogen appears strongly deshielded at 330 ppm.

In conclusion, a robust "get 2/pay 1" approach has been introduced to obtain two complementary HMBC spectra with the time usually required to record only one. The simultaneous acquisition of C-HMBC and N-HMBC spectra affords improved sensitivity ratios than individual acquisitions without sacrificing spectral quality, keeping minimum acquisition and processing set-ups and allowing simple implementation in automated protocols. The CN-HMBC experiment will find a general applicability in the structural analysis of a wide range of nitrogen-containing chemical compounds of diverse nature, namely synthetic organic and organometallic compounds, natural products, peptides, and nucleotides. The combined use of simultaneous ^{13}C and ^{15}N data will also facilitate the development of improved computer-assisted structure elucidation protocols.[16] Of particular interest could be also the application of the CN-HMBC experiment to other heteronuclei allowing, for instance, simultaneous recording of ^{1}H,^{13}C and ^{1}H,^{31}P(^{19}F) HMBC spectra in organophosphorus or organofluorine compounds. Much work is in progress on the development of improved CN-HMBC sequences and their application to structural characterization of molecules at natural abundance.

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Supporting Information Available: Experimental details and the program code (Bruker avance series) for the CN-HMBC pulse sequence described herein. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) Compared to regular C- and N-HMBC schemes, the overall duration of the CN-HMBC sequence is only modified for ^{13}C due to the t_1' period. For routine applications, a short $\Delta t_1'$ increment is strongly recommended in order to have a large enough spectral width covering the large dispersion in ^{15}N chemical shifts and to reduce the possible ^{13}C signal losses by additional relaxation. Folding for ^{15}N should be strongly avoided in the analysis of unknown structures because wrong chemical shift values could lead to wrong chemical interpretation.

(14) There are no special sample requirements to run CN-HMBC experiments, and the sensitivity is identical to that of the standard N-HMBC experiment. A triple-channel NMR system is the only hardware requirement.

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