Characteristics and a Convenient Measuring Method of ^1H - ^{15}N Heteronuclear Multiple Bond Correlation Spectroscopy

Qing Liu* and Xuerong Zhu

R & D Center of Chemistry, Huntsman Asia Pacific (Shanghai) Co., Ltd., Shanghai 200245, China

Some characteristics of ^1H - ^{15}N heteronuclear multiple bond correlation (HMBC) spectroscopy of an aliphatic amine was described. A measuring method of ^1H - ^{15}N HMBC spectroscopy at natural abundance was also introduced. ^1H - ^{15}N HMBC spectra can be recorded using the Bruker standard experiment, HMBCGP_15N. The optimal value of the acquisition parameter, CNST13, for this experiment ranges from 3 to 5. The measuring time is about 4 times longer than the ^1H - ^{13}C HMBC one.

Keywords ^1H - ^{15}N correlation, heteronuclear multiple bond correlation, NMR

Introduction

Nitrogen is an important element in biologically organic compounds, polymers, and biomacromolecules, etc. It has two isotopes (^{14}N and ^{15}N) in the nature. Despite very low natural abundance (0.37%) and relative NMR sensitivity (0.02, referred to ^{13}C) of ^{15}N , it is still preferred for detection owing to its favorable NMR properties (narrow NMR lines, and with the spin number $I=1/2$).^[1,2] In order to detect ^{15}N NMR signals efficiently, many efforts have been made, including direct detection methods (^{15}N labeling, polarization transfer and ^{15}N DEPT), and indirect detection methods (^1H - ^{15}N heteronuclear multiple bond correlation (HMBC) and ^1H - ^{15}N heteronuclear single bond correlation (HSQC)).^[1]

^1H - ^{15}N HMBC is the most successful method that detects ^{15}N NMR signals indirectly at natural abundance. It is a proton-detected experiment designed to obtain long-range (usually two- and three-bond) heteronuclear correlations between ^1H and ^{15}N via the scalar coupling constants. In many cases, ^1H - ^{15}N HMBC spectroscopy plays an important role in structural elucidation of nitrogenous constituents.^[3,4] Arguably, ^1H - ^{15}N HMBC began with a pair of poster presentations from laboratories in Japan and the United States in 1993.^[5] In recent years, many literatures^[6-8] have reported the applications of ^1H - ^{15}N HMBC spectroscopy in structural elucidation of nitrogenous compounds. However, the published reports introduced few characteristics of the ^1H - ^{15}N HMBC spectroscopy. To understand and utilize ^1H - ^{15}N HMBC spectroscopy well, the present work took 3-dimethylaminopropyl-2-hydroxypropylamine (Figure 1) as an example to describe some characteristics and a

convenient measuring method of ^1H - ^{15}N HMBC spectroscopy at natural abundance.

Figure 1 The structure of 3-dimethylaminopropyl-2-hydroxypropylamine.

Experimental

Materials

3-Dimethylaminopropyl-2-hydroxypropylamine, a colorless liquid, was synthesized in our group. It was dissolved in CDCl_3 (CIL, chloroform $D>99.8\%$, with 0.03% TMS) at a concentration of 30% (V/V) for NMR test. Its structure was established by 1D (^1H , ^{13}C and DEPT135) and 2D (HSQC and HMBC) NMR spectroscopy.

NMR spectroscopy

All the 1D and 2D NMR spectra were recorded on a Bruker AVANCE-III HD 500 MHz instrument at 298 K using a 5 mm PA BBO 500S1 BBF-H-D-05 Z SP probe. Data acquisition and process were conducted by the software Topspin 3.2. Chemical shifts were expressed in ppm, with tetramethylsilane as an internal standard.

^1H - ^{15}N HMBC experiments at natural abundance were obtained using the standard experiment named HMBCGP_15N in Topspin 3.2, with spectral widths of 5494.5 Hz in the ^1H dimension and 40548.3 Hz in the

* E-mail: sduqing@163.com; Tel.: 0086-021-33572721

Received October 21, 2015; accepted November 9, 2015; published online November 10, 2015.

Supporting information for this article is available on the WWW under www.genchemistry.org.

^{15}N dimension. The fid size $t_2 \times t_1$ was 2048×256 . The number of dummy scans and number of scans was 16 and 20, respectively.

The pulse sequence of the standard experiment HMBCGP_15N was shown in Figure 2, in which the ^1H 90° pulse created transverse magnetization firstly. After that, some of the transverse magnetization evolved into anti-phase magnetization at the end of d6 (d6 is delay for evolution of long range couplings). This anti-phase magnetization was converted into double quantum coherence by the first ^{15}N 90° pulse and evolved into chemical shifts during incremented delay, $2 \times \text{d0}$. During this time, the heteronuclear couplings and ^1H chemical shifts evolution were eliminated by a ^1H 180° pulse. In addition, three z -axis gradient pulses, 1 ms in length, with a gradient ratio of 70 : 30 : 50.1 were applied. The first two gradient pulses (70% and 30%) were placed on either side of the ^1H 180° pulse, while the final gradient pulse (50.1%) was after the last ^{15}N 90° pulse. The ^1H - ^{15}N HMBC data acquisition was in the magnitude mode with no decoupling.

Figure 2 The pulse sequence of HMBCGP_15N. D1 (2.0 s) was relaxation delay. D6 (0.1 s) was delay for evolution of long range couplings. D0 (3.0 μs) was incremented delay. G1, G2 and G3 were three z -axis gradient pulses with the value of 70%, 30% and 50.1%, respectively.

The detection of ^1H - ^{15}N HMBC correlation signals mainly depended on the value of an acquisition parameter CNST13, which represented long range coupling constants of $J_{\text{N-H}}$. If the ^1H - ^{15}N multiple-bond coupling constants $^nJ_{\text{N-H}}$ ($n \geq 2$) of the test samples are smaller than CNST13, the corresponding ^1H - ^{15}N correlations won't be observed. In present work, CNST13 was set from 1 to 7 to discuss the characteristics of ^1H - ^{15}N HMBC spectroscopy.

Results and Discussion

The ^1H and ^{13}C NMR data (Table 1) of 3-dimethylaminopropyl-2-hydroxypropylamine were assigned based on HSQC and HMBC experiments.

With regard to nitrogenous compounds, the ^1H - ^{15}N multiple-bond coupling constants, $^nJ_{\text{N-H}}$, are strongly dependent on the orientation of the protons with respect to the nitrogen lone pair of electrons. In many cases,

Table 1 ^1H (500 MHz) and ^{13}C (125 MHz) NMR data of 3-dimethylaminopropyl-2-hydroxypropylamine in CDCl_3

Position	δ_{H}	δ_{C}
1	0.70 (d, $J=6.0$ Hz, 3H)	20.2, 20.3 (CH_3)
2	3.39 (m, 1H)	63.5, 65.2 (CH)
3	1.93 (dd, $J=4.5, 14.0$ Hz, 1H) 1.95 (m, 1H)	62.1, 63.2 (CH_2)
4	2.22 (t, $J=6.8$ Hz, 2H)	52.0, 52.7 (CH_2)
5	1.19 (t, $J=6.8$ Hz, 2H)	24.0, 24.3 (CH_2)
6	1.95 (m, 2H)	56.2, 56.6 (CH_2)
7	1.79 (s, 3H)	44.7 (CH_3)
8	1.79 (s, 3H)	44.7 (CH_3)

$^3J_{\text{N-H}} > ^2J_{\text{N-H}} > ^4J_{\text{N-H}}$.^[3] Actually, most $^3J_{\text{N-H}}$ and $^2J_{\text{N-H}}$ values are less than 10 Hz,^[7] and $^2J_{\text{N-H}}$ may be a large negative or a small positive value.^[9] In addition, ^1H - ^{15}N coupling constants often span a very wide range of values in the same molecule, making the detection in a single experiment problematic.^[7] Therefore, CNST13 was set from 1 to 7 to discuss the characteristics of ^1H - ^{15}N HMBC spectroscopy in present work.

The defaulted CNST13 value in Topspin 3.2 is 5. Under this defaulted condition, all the 3J and part of 2J ^1H - ^{15}N correlations could be observed (Figure 3). More 2J and some 4J ^1H - ^{15}N correlations would be present if CNST13 became smaller (be reduced to 2, for example). However, CNST13 value can not be too small, because the resulting length of the delay will cause significant loss of signal via T2 relaxation processes.^[7] When CNST13 value was set to be 1, some signals disappeared or became weaker. Large CNST13 value is also disadvantageous to observation of ^1H - ^{15}N HMBC correlations. 2J ^1H - ^{15}N correlations became weaker once the value of CNST13 was changed to 7.

Figure 3 The ^1H - ^{15}N HMBC correlations of 3-dimethylaminopropyl-2-hydroxypropylamine in CDCl_3 .

Although small CNST13 value could bring more ^1H - ^{15}N correlation signals, it also would cost more acquisition time due to the loss of signal via relaxation processes. When CNST13 value was reduced to 2 from 5, the total acquisition time had to be changed from 40

NOTE

min to 170 min to get a spectrum with the same quality. Considering observation of 2J correlations and acquisition time, optimal values for CNST13 could be in the range of 3–5.

By the way, it is interesting that despite correlations of H-7 and H-8 to N-2' were 2J correlations, the detection of these 2J correlations seemed not to be affected by CNST13 value. They could be observed easily no matter how CNST13 varied from 1 to 7. This may owe to large coupling constants between H-7/H-8 and N-2'.

Besides CNST13 value, a proper measuring time (number of scans) is also important to obtain a ^1H - ^{15}N HMBC spectrum with high quality. It's reported that the ^1H - ^{15}N HMBC experiment is only about three times less sensitive than the ^1H - ^{13}C HMBC one, leading to the measuring time of a ^1H - ^{15}N HMBC spectrum is about 6 times longer.^[10] As a matter of experience, the measuring time of a ^1H - ^{15}N HMBC experiment can be about 4 times longer than the ^1H - ^{13}C HMBC one, under the condition that CNST13 value is 5.

Conclusions

^1H - ^{15}N HMBC spectroscopy has become a powerful tool in the structural elucidation of nitrogenous compounds, including polymers, natural products, and biomacromolecules, etc. For aliphatic amines, 3J ^1H - ^{15}N correlations are easier observed than the 2J ones (except for 2J methyl to nitrogen correlations). ^1H - ^{15}N HMBC

spectroscopy at natural abundance can be recorded conveniently using the Bruker standard experiment, HMBGP_15N. The optimal value of CNST13 (an acquisition parameter) for HMBGP_15N ranges from 3 to 5. The measuring time is about 4 times longer than the ^1H - ^{13}C HMBC one.

References

- [1] Marek, R.; Lycka, A. *Curr. Org. Chem.* **2002**, *6*, 35.
- [2] Marek, R.; Lycka, A.; Kolehmainen, E.; Sievanen, E.; Tousek, J. *Curr. Org. Chem.* **2007**, *11*, 1154.
- [3] Philbrook, A.; Blake, C. J.; Dunlop, N.; Easton, C. J.; Keniry, M. A.; Simpson, J. S. *Polymer* **2005**, *46*, 2153.
- [4] McDonnell, P. A.; Gauthier, A. G.; Ferro, M. T. *Magn. Reson. Chem.* **1998**, *36*, 35.
- [5] Martin, G. E.; Williams, A. J. *Annu. Rep. NMR Spectro.* **2005**, *55*, 1.
- [6] Martin, G. E.; Crouch, R. C. *J. Nat. Prod.* **1996**, *59*, 2.
- [7] Kline, M.; Cheatham, S. *Magn. Reson. Chem.* **2003**, *41*, 307.
- [8] Mei, Q.; Wang, Y. N.; Zhao, M.; Liu, X. Y.; Peng, S. Q.; Wang, F. P. *Chin. Chem. Lett.* **2015**, *26*, 804.
- [9] Martin, G. J.; Martin, M. L.; Gouesnard, J. P. *^{15}N -NMR spectroscopy*, Vol. 18, Eds.: Diehl, P.; Fluck, E.; Kosfeld, R., Springer Science & Business Media, Berlin, **2012**, p. 194.
- [10] Köck, M.; Junker, J.; Lindel, T. *Org. Lett.* **1999**, *1*, 2041.