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Gradient-Enhanced HSQC Experiments for Phase-Sensitive Detection of Multiple Bond Interactions

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Abstract: Observation of remote connectivities and determination of long range coupling constants by ^{1}H-X (^{13}C, ^{15}N and ^{77}Se) gradient-enhanced phase-sensitive HSQC is reported.

The long range heteronuclear NMR techniques correlating the chemical shifts of protons and X-nuclei, such as ^{13}C or ^{15}N, supply valuable data for molecular structure determination. The initial experiments[1,2] based on direct observation of X resonances have been almost exclusively replaced by proton-detected variants[3]. These so called '*inverse*' experiments bring dramatic increase in sensitivity since highly sensitive ^{1}H nuclei are employed to monitor the spectral parameters of X nuclei.

Recently, performance of the inverse-detected experiments has been significantly improved by the introduction of pulsed field gradients (PFG)[4,5,6]. The utilization of PFG proved to be extremely useful in applications optimized to detect the long range scalar interactions. Gradient-enhanced experiments have particularly beneficial effects in eliminating the t_1-noise, improving the suppression of solvent signals, removing spectral artifacts and facilitating observation of very weak interactions across several bonds (e.g. ^{4}J).

Two-dimensional (2D) heteronuclear multiple-bond correlation (HMBC) experiment[7] optimized for different heteronuclei[8] can be applied to trace remote connectivities. The values of heteronuclear coupling constants can be extracted from the splitting of doublets in 2D spectra. However, a magnitude mode presentation of gradient-enhanced HMBC spectra[9] precludes the measurements of small coupling constants since in most cases only unresolved doublets[10] can be observed.

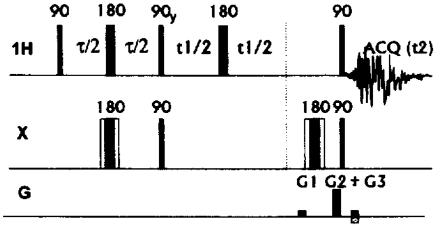

Figure 1

In order to circumvent limitations of magnitude mode HMBC we have applied the heteronuclear single-quantum correlation experiment[11-13] (HSQC). The phase sensitive version which allows to extract the values of scalar couplings is shown in Figure 1.

The pulse sequence is a straightforward modification of a non-refocused HSQC experiment. This technique employs an INEPT step[14] to transfer the proton magnetization to the J-coupled heteronuclei. Composite $(\pi)_X$ pulse is applied in the middle of an INEPT transfer in order to minimize pulse imperfections and the evolution delay τ is set to match approximately $1/2^{m}J$, where ^{m}J represents the active long range scalar interaction. At the end of the INEPT step only the heteronuclear single-quantum magnetization $2H_zX_y$ is generated. This magnetization is labeled by the heteronuclear chemical shift during the t_1 evolution. Two phase encoding gradients are applied around the π_X pulse at the end of the t_1 period in order to suppress the X chemical shift evolution during the gradient dephasing. The amplitude of gradient dephasing is given by difference $|G_2 - G_1|$. The small symmetrical G_1 gradient around the π_X pulse substitutes the EXORCYCLE phase cycle[15] and removes the echo imperfections.[16] The proton antiphase magnetization detected during the acquisition evolves as $\sin(\pi.^{m}J.t_2)$ giving the echo with a maximum signal amplitude at $t_2 \sim \tau$. The receiver is opened immediately after the back transfer of magnetization to ^1H nuclei by a pair of $\pi/2$ X and ^1H pulses and the phase decoding gradient G_3 is applied during t_2. For the ^1H - X spin system, the complete phase decoding of two alternative coherence transfer pathways $X^- \Rightarrow X^+ \Rightarrow H^-$ and $X^+ \Rightarrow X^- \Rightarrow H^-$ during the t_2 detection[17] is obtained if:

$$(G_2 - G_1) = \pm \gamma_H / \gamma_X G_3 \qquad (1)$$

and $G_2 > G_1$.

The amplitude of the decoding gradient G_3 is altered in subsequent acquisitions. Their results are stored at separate memory locations and processed according to the echo-antiecho protocol.[18] This generates spectra which can be phased to pure absorption both in the F_1 and F_2 frequency domain.

Application of the gradient enhanced phase-sensitive multiple bond HSQC experiment (GSQMBC-Gradient-enhanced Single Quantum Multiple Bond Correlation) for determination of coherence transfer pathways as well as observation of small long-range coupling constants ^{m}J is demonstrated for an acylselenourea derivative 1[19] (Figure 2).

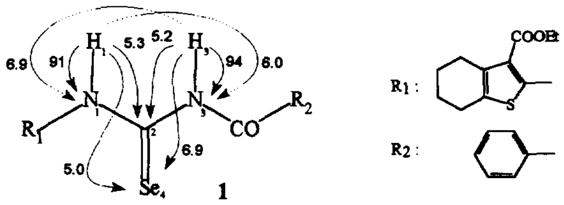

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Figure 2

The scalar interactions of amino protons with ^{13}C, ^{15}N and ^{77}Se nuclei across two and three bonds which characterize the stereochemical arrangement of the acylselenourea compound 1 were extracted from the GSQMBS spectra.

The measurement of small long-range coupling constants in substituted selenourea 1 is complicated by the fact that protons of interest are bound to nitrogen. At natural abundance the nitrogen atom is mostly ^{14}N isotope (99.63%). The quadrupolar interaction of this nucleus causes relatively fast relaxation of the directly bonded protons and rapid signal decay. Figure 3 compares sections of the magnitude mode ^1H-^{15}N HMBC and phase-sensitive ^1H-^{15}N HSQC spectra of compound 1.

The spectra show one-bond interactions (${}^{1}J_{H1,N1}$ = 91 Hz) of H1 proton (15.12 ppm) to nitrogen N1 (156.2 ppm) and three-bond connectivities of H1 to N3 (164.8 ppm). While the proton linewidth for a single bond correlation H1-N1 is narrow as a result of the ${}^{1}H$-${}^{15}N$ pairs selection, the signal linewidth of H1-N3 crosspeaks is enlarged by fast quadrupolar ${}^{1}H$-${}^{14}N$ one-bond relaxation leading to the unresolved crosspeak in the HMBC spectrum (Figure 3a). In contrast, the HSQC spectrum (Figure 3b) shows clearly the antiphase doublet from which the corresponding three-bond coupling constant ${}^{3}J_{H1,N3}$ = 6.0 Hz was obtained. The positive and negative signals are plotted with the same contour pattern.

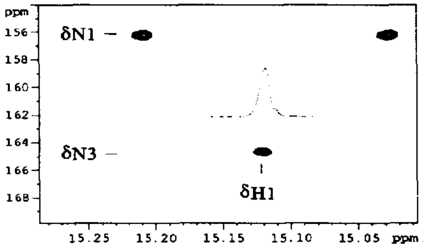

Figure 3a

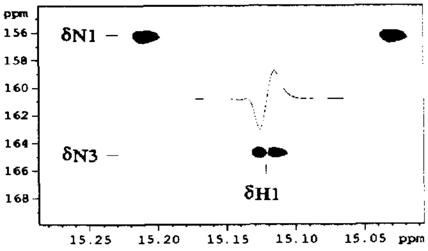

Figure 3b

The value of ${}^{n}J$ measured directly from the spectrum must be interpreted with caution. Incomplete separation of the antiphase components leads to a partial overlap and the obtained value overestimates the real J.[20]

The GSQMBC experiment was succesfully applied also to detect the three-bond ${}^{1}H$-${}^{77}Se$ and two and three-bond ${}^{1}H$-${}^{13}C$ interactions. Figure 4 shows a partial ${}^{1}H$-${}^{77}Se$ HSQC spectra of compound 1 for both protons H1 and H3. As in the case of three-bond ${}^{1}H$-${}^{15}N$ interactions the upper limiting values for ${}^{3}J_{H1,Se4}$ = 5.0 Hz and ${}^{3}J_{H3,Se4}$ = 6.9 Hz were extracted from the spectra.

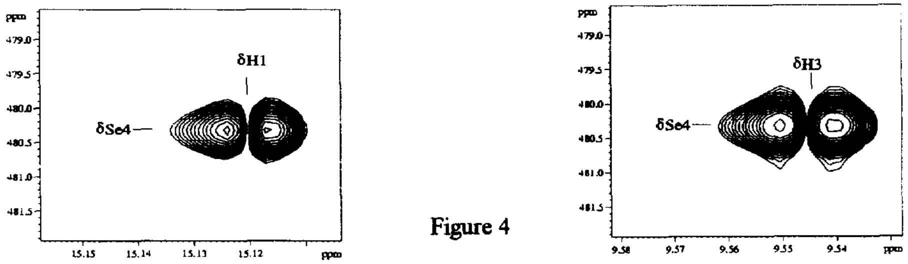

Figure 4

The measured values of heteronuclear coupling constants are summarized in Figure 2.

The GSQMBC sequence (Figure 1) has been implemented without a low-pass J-filter because clear evidence of one bond interactions proved to be helpful in signal assignments. However, when necessary a low-pass J-filter[21] can be incorporated to suppress the one-bond correlations in order to simplify the crowded regions of 2D spectra.

As demonstrated, the phase-sensitive GSQMBC technique represents a preferential experiment for the study of heteronuclear long-range coupling pathways and determination of corresponding coupling constants. The method can be used in conformational studies since heteronuclear three-bond coupling constants allow to extract approximate values of dihedral angles in cases when the Karplus relation[22] is known.

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8. NMR spectra were recorded on a Bruker Avance DRX-500 spectrometer. The ^{13}C and ^{1}H spectra were referenced to tetramethylsilane used as internal standard. ^{77}Se chemical shifts were referenced to 85% H_2SeO_3 (1282 ppm) and $SeOCl_2$ (1479 ppm) and ^{15}N chemical shifts to liquid ammonia (0 ppm) used as external standards. Experiments were performed with inverse tripleresonance probehead (^{1}H{^{13}C/BB}) equipped with a z-gradient coil.
   HMBC sequence: D1-90°(^{1}H)-D6-90°(^{13}C)-t_1/2-G1-D16-180°(^{1}H)-G2-D16-t_1/2-90°(^{13}C)-G3-D16--ACQ(t_2), D16 = 100 ms, G = 1ms, D6 = 60 - 120 ms, ^{1}H-^{15}N correlation: D1 = 2.7s, G1:G2:G3 = 42:18:30G/cm, ^{1}H-^{77}Se correlation: D1 = 2.5s, G1:G2:G3 = 36:24:23.4G/cm
   GSQMBC (sequence on Figure 1): D16 = 100 ms, G = 1ms, ^{1}H-^{13}C correlation: D1 = 2.2s, G1:G2:G3 = 12:36:±6G/cm, ^{1}H-^{15}N correlation: D1 = 2.7s, G1:G2:G3 = 4.8:52.8:±4.8G/cm
   ^{1}H-^{77}Se correlation: D1 = 2.5s, G1:G2:G3 = 12:43.6:±6G/cm, ^{1}H NMR: 9.48 (H-3), 15.12 (H-1), ^{13}C NMR: 174.47 (C-2), ^{15}N NMR: 156.2 (N-1), 164.8 (N-3), ^{77}Se NMR: 480 (Se-4).
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