Precise measurement of long-range heteronuclear coupling constants by a novel broadband proton-proton - decoupled CPMG-HSQMBC method

István Timári,[a] Tünde Z. Illyés,[b] Ralph W. Adams,[c] Mathias Nilsson,[c] László Szilágyi,[b] Gareth A. Morris,[c] and Katalin E. Kövér*[a]

Abstract: We present a broadband proton-proton - decoupled CPMG-HSQMBC method for the precise and direct measurement of long-range heteronuclear coupling constants. The Zangger-Sterk - based homodecoupling scheme reported here efficiently removes unwanted proton-proton splittings from the heteronuclear multiplets, allowing the desired heteronuclear couplings to be determined simply by measuring frequency differences between singlet maxima in the resulting spectra. The pseudo 1D/2D pulse sequences proposed have been tested on nucleotides, a metal complex incorporating P-heterocycles, and diglycosyl-(di)selenides, as well as on other carbohydrate derivatives, for the extraction of $^{n}J(^{1}H, ^{31}P)$, $^{n}J(^{1}H, ^{77}Se)$ and $^{n}J(^{1}H, ^{13}C)$ values, respectively.

Introduction

Long-range heteronuclear coupling constants ($^{n}J_{XH}$) provide invaluable tools for stereochemical and conformational analysis of synthetic organic molecules[1] and natural products,[2] and complement the information gained from proton-proton coupling constants and NOE data.[3] Even though many different approaches to their measurement have been proposed over the last two decades,[4-5] measurement of $^{n}J_{XH}$ values is still not straightforward and is therefore relatively unexploited in structural studies of molecules.

Among the methods reported in the literature, HETLOC[6-7] and HSQC-TOCSY[8-11] experiments are particularly useful for the measurement of heteronuclear multiple-bond couplings of protonated heteronuclei, but they fail for non-protonated (e.g. quaternary C) centers, or when proton-proton TOCSY transfer is not efficient. In contrast, HMBC[12-14] and HSQMBC[15] methods and their variants are applicable regardless of the protonation state of the heteronucleus. However, a common drawback of HMBC- and HSQMBC-type approaches is that during the long (ca. 70-90 ms) coupling evolution period the homonuclear proton-proton and the proton-heteronucleus long-range coupling interactions evolve together, resulting in mixed-phase signals in the resultant spectra. Therefore, extraction of the desired heteronuclear coupling constants often requires the use of complex fitting procedures; at worst, extraction of the coupling values of interest may even be prevented where multiplets are severely distorted. To circumvent this limitation of the HSQMBC method, several modifications have been introduced into the long-range coupling-matched INEPT component of the sequence, such as application of CPMG pulse trains,[16-18] selective and band-selective 180° proton pulses[19-20] or a perfect echo element.[21] However, even in these amended variants the resulting HSQMBC peaks appear with complex multiplet patterns in which the undesired proton-proton splittings are superimposed on the antiphase doublets originating from the active heteronuclear coupling interactions. Thus the evolution of proton-proton couplings during acquisition, resulting in complex antiphase multiplets, can impede the extraction of the heteronuclear coupling constants.

Recently it has been shown that proton-proton splittings can be eliminated from HSQMBC spectra by applying band-selective homonuclear decoupling to spectral regions with non-mutually coupled proton sites.[22] Application of this scheme is, however, limited to molecules with specific types of structure, for example peptides.[22] Homonuclear broadband decoupled ^{1}H experiments have also been used for the measurement of heteronuclear coupling constants for compounds containing highly abundant heteronuclei.[23-24]

Here we report a novel broadband proton-proton - decoupled CPMG-HSQMBC method for the simple and precise measurement of long-range heteronuclear coupling constants. In the experiment proposed the undesired proton-proton splittings are eliminated with the aid of a broadband homodecoupling scheme based on the Zangger-Sterk principle;[25] as a result, only evolution of heteronuclear couplings is active during acquisition. The desired multiple-bond heteronuclear couplings can thus be extracted simply by measuring the frequency differences between the peaks of pure antiphase doublets. In addition, the relative signs of coupling constants can be determined from the characteristic sign pattern (*up*/*down* or *down*/*up*) of the antiphase signals.

Results and Discussion

Pure shift methods which suppress the effects of proton-proton scalar couplings in the directly detected proton dimension provide simplified spectra and increased resolution, and have attracted considerable attention in recent years.[26-44] Common broadband proton-proton decoupling methods include those

[a] I. Timári, Prof. Dr. K. E. Kövér
Department of Inorganic and Analytical Chemistry
University of Debrecen
Egyetem tér 1, H-4032 Debrecen, Hungary
E-mail: kover@science.unideb.hu
[b] Dr. T. Z. Illyés, Prof. Dr. L. Szilágyi
Department of Organic Chemistry
University of Debrecen
Egyetem tér 1, H-4032 Debrecen, Hungary
[c] Dr. R. W. Adams, Dr. M. Nilsson, Prof. Dr. G. A. Morris
School of Chemistry
University of Manchester
Oxford Road, Manchester, M13 9PL, United Kingdom

Supporting information for this article is given via a link at the end of the document.

based on the bilinear rotation decoupling (BIRD)[32-37] and Zangger-Sterk (ZS) pulse sequence modules.[26-31] The former utilizes an isotope selection approach: depending on the relative phases of the individual proton pulses of BIRD modules,[45] protons either directly attached, or not attached, to isotopically dilute spins (e.g. ^{13}C, ^{15}N) can be selectively and independently inverted. The ZS method[25] uses spatially- and frequency-selective excitation, combining a selective 180° proton pulse with a weak magnetic field gradient.

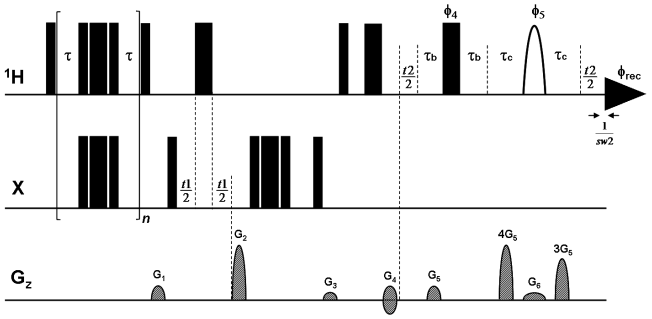


Figure 1. Pulse sequence scheme of the broadband proton-proton - decoupled CPMG-HSQMBC experiment designed for the measurement of long-range heteronuclear coupling constants. Narrow and wide filled bars correspond to 90° and 180° pulses respectively, with phase *x* unless indicated otherwise. The selective shaped proton pulse is shown as a half-ellipse. ϕ_1 is incremented according to XY-16 cycles within the CPMG sequence, thus *n* should ideally be adjusted to a multiple of 16. Other phases are ϕ_2 = *y*; ϕ_3 = *x, -x*; ϕ_4 = *x, x, -x, -x*; ϕ_5 = *x, x, x, x, y, y, y, y*; and ϕ_{rec} = *x, -x, -x, x, -x, x, x, -x*. Delays are set as follows: τ = 120-150 μs, τ_a = 1/(4*sw2), τ_c = 1/(4*sw2) – 4/sw. Coherence order selection and echo-antiecho phase sensitive quadrature detection in the X-dimension are achieved with gradient pulses G_2 and G_4 in the ratio 80 : 20.1 for ^{13}C, 80 : 32.3846 for ^{31}P and 80 : 15.257 for ^{77}Se, respectively. Purging gradient pulses G_1 and G_3 are set to 19% and 10% of maximum gradient strength (50 G/cm). Coherence selection gradient pulses used in the extra proton-proton - decoupled dimension have G_5 = 18%. Sine bell shaped gradient pulses of 1 ms duration are utilized, followed by a recovery delay of 200 μs. The slice-selection gradient (G_6) is adjusted for each molecule as reported in the legends to the respective figures.

Here, we propose a broadband proton-proton - decoupled CPMG-HSQMBC experiment (Figure 1) which utilizes an improved version of the Zangger-Sterk broadband homodecoupling scheme. As shown in the pulse sequence of Figure 1, broadband proton decoupling in the directly detected proton dimension is achieved by replacing the conventional free induction decay (FID) acquisition of the CPMG-HSQMBC sequence with a second evolution time, t_2, during which a hard 180° proton pulse and a weak gradient field under a selective 180° proton pulse are applied in succession, followed by acquisition of a chunk of FID $s(t_3)$. The weak gradient field combined with the selective 180° proton pulse is used to restrict the measurement of the signal from each different chemical shift in the spectrum to a different horizontal slice through the sample. The combination of selective and non-selective 180° proton pulses then ensures that all protons which are off-resonance are inverted while the on-resonance protons (and the undisturbed heteronuclei) remain unaffected. Consequently, the net effect is to allow the continuous evolution of the proton chemical shift and the heteronuclear coupling throughout t_2, but to refocus the evolution of the undesired proton-proton couplings at the midpoint of the acquisition of a FID chunk. Because proton-proton couplings evolve much more slowly than chemical shifts, FID chunks $s(t_3)$ can be typically acquired with a duration of 10 - 25 ms, matched to the increment, 1/sw2, used for the second evolution time (t_2). During processing, prior to regular 2D FT a pseudo-2D dataset (interferogram) is constructed by concatenating all the data chunks recorded, to give a synthetic FID without homonuclear *J* modulation.

In all Zangger-Sterk type experiments, however, including our new method, there is a trade-off between the sensitivity, the minimum frequency difference to be decoupled, and the range of chemical shifts to be covered. Typically, the sensitivity of these proton-decoupled experiments is ca. 1-10 % of that of the conventional analogue.[46] The actual sensitivity loss depends on the choice of experimental parameters for slice selection, which in turn depend on the nature of the spin systems involved. For efficient homonuclear decoupling, the selective pulse should be selective enough to affect only one coupling partner. However a soft pulse with narrow bandwidth generates signal from only a thin slice of the sample, hence reducing the sensitivity of the experiment. The range of chemical shifts to be decoupled determines the strength of the gradient required, so increasing the shift range again reduces the slice thickness and hence the sensitivity. Thus the sensitivity of a ZS experiment is directly proportional to the bandwidth of the selective pulse, and inversely proportional to the strength of the slice selection gradient. In practice, these two parameters should be carefully chosen for a given sample, for example with the help of the much quicker 1D pure shift (ZS decoupled) ^1H experiment.[27]

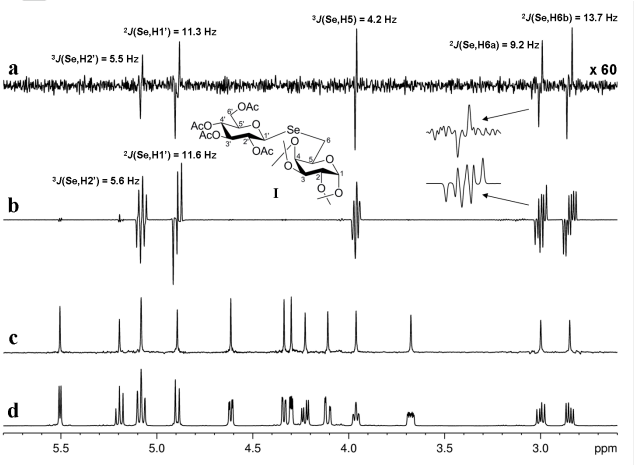


Figure 2. Comparison of CPMG-HSQMBC spectra obtained for a diglycosyl-selenide (**I**), with (a) and without (b) broadband proton-proton decoupling. Measurement times were 5.9 h (a) and 45 min (b), respectively. (The structure, with numbering, is shown in the inset.) The homonuclear decoupled pseudo 1D spectrum (a) was collected using the sequence of Figure 1 with the incremented delay t_1 replaced by a constant delay of 3 μs. Representative ZS-based pure shift ^1H (c) and normal ^1H NMR spectra (d) are also shown. All spectra in Figure 2 were recorded with spectral widths = 6.0371 ppm. In the broadband proton-proton - decoupled spectra (a,c) an RSNOB selective 180° proton pulse[47] of duration 46.64 ms and bandwidth 50 Hz under a slice selection gradient (G6) of 1 % of the maximum gradient strength was used.

These spectra (a,c) were acquired with number of t_2 increments (i.e. number of FID chunks) = 32, duration of FID chunk = 16.56 ms, number of complex data points of constructed FID in ^{1}H dimension = 3200 , relaxation delay = 2 s, number of scans = 128 for spectrum (a) and 4 for spectrum (c). Spectrum (b) was collected with number of complex data points = 4096, relaxation delay = 1.7 s and with number of scans = 1024, using the conventional CPMG-HSQMBC sequence.[17] The HSQMBC experiments (a,b) were recorded with 81.7 ms of heteronuclear coupling evolution during the initial CPMG-INEPT step.

To validate the performance of the new method, we first tried a pseudo one-dimensional (1D) version of the proton-decoupled CPMG-HSQMBC sequence on simple model compounds containing only one highly (^{31}P: 391 times more sensitive than ^{13}C) or one moderately (^{77}Se: 3.15 times more sensitive than ^{13}C) sensitive heteronucleus, but featuring an extensive set of mutually coupled protons. As a first example, Figure 2 shows the standard ^{1}H, pure shift (PS) ^{1}H, standard CPMG-HSQMBC[17] and broadband proton-proton - decoupled CPMG-HSQMBC spectra of a diglycosyl-selenide model (**I**) (see structure with numbering of atoms in Figure 2). It can be seen that in the traditional CPMG-HSQMBC multiplets (Figure 2b) the many in-phase proton-proton splittings severely compromise, in several cases, extraction of the multiple-bond ^{1}H-^{77}Se coupling constants from the complex (in- and antiphase) multiplets. In contrast, the pure antiphase doublets of the broadband proton-proton - decoupled CPMG-HSQMBC spectrum shown in Figure 2a allow the measurement of all desired heteronuclear couplings with ease and with high precision. For comparison, values of coupling constants were extracted from both the traditional proton-coupled and the new proton-decoupled HSQMBC multiplets. The coupling constants obtained by the two different methods (as given above the corresponding multiplets in Figures 2, 3 and 4) agree within experimental error, confirming that the proton-proton decoupling sequence applied during acquisition has no undesired effect on the measured multiplet splittings.

Based on our previous studies with other selenoglycosides, such coupling data are highly valuable and represent a promising tool for the assessment of the glycosidic conformation around the C(1)-Se bond[48] or for the unambiguous stereospecific assignment of diastereotopic CH_2-protons next to Se.[49]

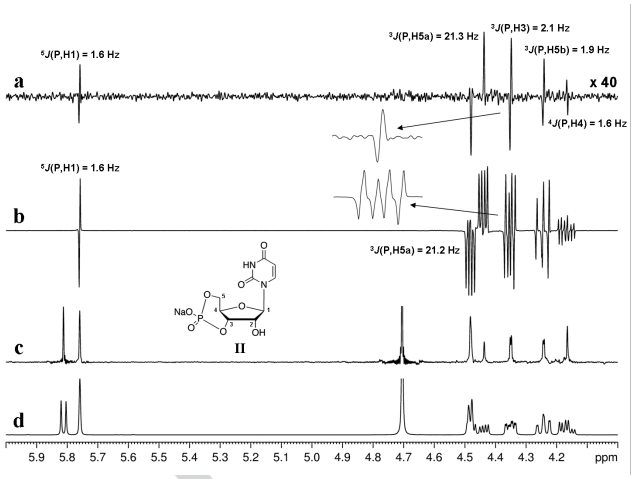


Figure 3. Comparison of CPMG-HSQMBC spectra obtained for cUMP (**II**), with (a) and without (b) broadband proton-proton decoupling. Measurement times were 1.5 h (a) and 10 min (b), respectively. (The structure, with numbering, is shown in the inset.) The homonuclear decoupled pseudo 1D spectrum (a) was collected using the sequence of Figure 1 with the incremented delay t_1 replaced by a constant delay of 3 μs. Representative ZS-based pure shift ^{1}H (c) and normal ^{1}H NMR spectra (d) are also shown. Spectra (a,c,d) were recorded with spectral widths = 6.0371 ppm. In the cases of the broadband proton-proton - decoupled spectra (a,c) an RSNOB selective 180° proton pulse[47] of duration 93.28 ms and bandwidth 25 Hz under a slice selection gradient (G6) of 1% of the maximum gradient strength was used. These spectra (a,c) were acquired with number of t_2 increments (i.e. number of FID chunks) = 32, duration of FID chunk = 16.56 ms, number of complex data points of constructed FID in ^{1}H dimension = 3200, relaxation delay = 1.7 s, number of scans = 32 for spectrum (a) and 8 for spectrum (c). Spectrum (b) was collected with spectral width = 9.9774 ppm, number of complex data points = 16384, relaxation delay = 1.7 s and with number of scans = 128 using the conventional CPMG-HSQMBC sequence.[17] The HSQMBC experiments (a,b) were recorded with 81.7 ms of heteronuclear coupling evolution during the initial CPMG-INEPT step.

Test measurements were run to assess the scope of our method for the determination of other long-range heteronuclear coupling constants such as $^nJ(^1H,^{31}P)$. For cUMP (**II**) (see structure with numbering of atoms in Figure 3), a biologically relevant nucleotide, all long-range ^{1}H-^{31}P coupling constants could be determined from the broadband proton-proton - decoupled CPMG-HSQMBC spectrum simply by measuring the frequency differences between the peaks of pure antiphase doublets (Figure 3a), whereas the analysis of the conventional CPMG-HSQMBC multiplets (Figure 3b) is not straightforward. Figure 3a also illustrates that multiple-bond heteronuclear coupling constants ranging between 1.6 Hz and 21.3 Hz can be measured in a single experiment using our new method. These results also clearly demonstrate that the proposed pulse sequence shown in Figure 1, together with the gradient based coherence selection scheme, efficiently removes undesired coherences arising from any mismatch between the duration of the CPMG-INEPT delay and $^nJ(^1H, X)$. It has already been well demonstrated in the literature that ZS-based broadband proton-proton decoupling schemes can handle highly complex proton-proton coupled spin networks, and this paves the way for the applicability and utility of our approach for studying more

complex systems, as is nicely illustrated in the examples shown in Figures 2 and 3.

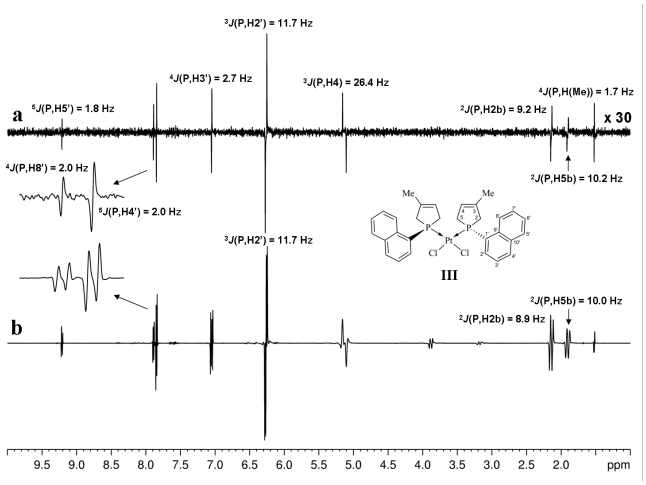


Figure 4. Comparison of CPMG-HSQMBC spectra obtained for a Pt complex incorporating P-heterocycles (**III**), with (a) and without (b) broadband proton-proton decoupling. Measurement times were 3.3 h (a) and 13 min (b), respectively. (The structure, with numbering, is shown in the inset.) The homonuclear decoupled pseudo 1D spectrum (a) was collected using the sequence of Figure 1 with the incremented delay t_1 replaced by a constant delay of 3 μs, and using an RSNOB selective 180° proton pulse[47] of duration 23.32 ms and bandwidth 100 Hz under a slice selection gradient (G6) of 1.6 % of the maximum gradient strength. This spectrum (a) was recorded with spectral width = 9.9774 ppm, number of t_2 increments (i.e. number of FID chunks) = 32, duration of FID chunk = 20.04 ms, number of complex data points of constructed FID in ^{1}H dimension = 6400, relaxation delay = 1.7 s and with number of scans = 96. Spectrum (b) was acquired with spectral width = 9.9774 ppm, number of complex data points = 8192, relaxation delay = 2 s and with number of scans = 256 using the conventional 1D CPMG-HSQMBC sequence.[17] The HSQMBC experiments (a,b) were recorded with 52.7 ms of heteronuclear coupling evolution during the initial CPMG-INEPT step.

Next, the usefulness of the proposed method is further illustrated with a metal complex incorporating P-heterocycles (**III**) (Figure 4, see structure[50] in the inset). The broadband proton-proton - decoupled CPMG-HSQMBC spectrum clearly demonstrates that — if necessary — signals for an extensive range of chemical shifts can be recorded in a single experiment, and splittings measured, by suitably adjusting the strength of the slice selection gradient (Figure 4a).

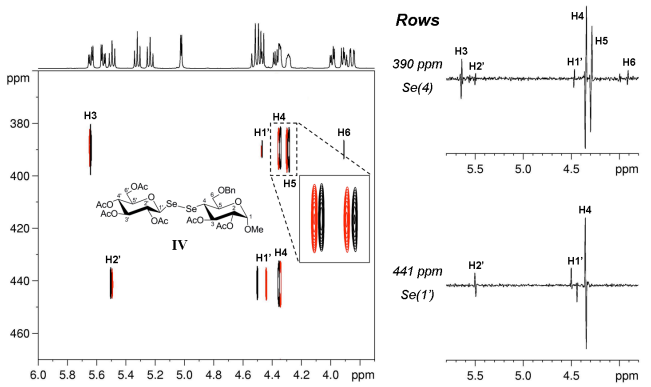


Figure 5. Representative broadband proton-proton - decoupled 2D CPMG-HSQMBC spectrum of a diglycosyl-diselenide (**IV**). (The structure, with numbering, is shown in the inset.) The extracted selenium traces shown next to the 2D spectrum nicely illustrate that the proposed method results in clean, pure absorptive antiphase doublets with splittings due solely to the desired multiple-bond heteronuclear couplings. The normal ^{1}H spectrum can be seen above the 2D contour plot. The broadband proton-proton - decoupled CPMG-HSQMBC spectrum was recorded using an RSNOB selective 180° proton pulse[47] of duration 46.64 ms and bandwidth 50 Hz under a slice selection gradient (G6) of 0.5% of the maximum gradient strength. The spectrum was acquired at 308 K in experiment time of 18.3 h with spectral widths in the ^{1}H (^{77}Se) dimension = 9.9774 (140.0) ppm, number of t_1 increments = 32, number of t_2 increments (i.e. number of FID chunks) = 16, duration of FID chunk = 16.56 ms, number of complex data points of constructed FID in ^{1}H dimension = 1600, relaxation delay = 1.7 s, number of scans = 48 and with duration of long-range heteronuclear coupling evolution = 81.7 ms.

The pseudo two-dimensional (2D) version of the broadband proton-proton - decoupled CPMG-HSQMBC experiment has been tested on a diglycosyl-diselenide (**IV**) featuring a unique Se-Se bond in the interglycosidic bridge (see structure with numbering of atoms in Figure 5). The 1D traces extracted at the corresponding Se-chemical shifts in Figure 5 nicely illustrate that the proposed 2D experiment results in clean, pure absorptive antiphase doublets with splittings arising solely from multiple-bond heteronuclear couplings, allowing direct and precise measurement of $^nJ(^1H, ^{77}Se)$ for molecules with more than one Se site.

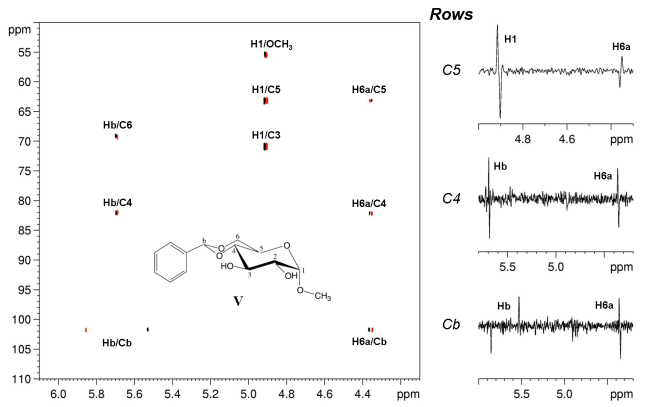


Figure 6. Partial contour plot of the broadband proton-proton - decoupled 2D CPMG-HSQMBC spectrum of Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**V**). (The structure, with numbering, is shown in the inset.) The extracted carbon traces shown next to the 2D spectrum show pure absorptive antiphase doublets with splittings due solely to the heteronuclear couplings. The spectrum was recorded using an RSNOB selective 180° proton pulse[47] of duration 46.64 ms and bandwidth 50 Hz under a slice selection gradient (G6) of 1% of the maximum gradient strength. The spectrum was acquired in experiment time of 38.7 h with spectral widths in the ^{1}H (^{13}C) dimension = 6.0370 (80.0) ppm, number of t_1 increments = 200, number of t_2 increments (i.e. number of FID chunks) = 16, duration of FID chunk = 21.12 ms, number of complex data points of constructed FID in ^{1}H dimension = 2048, relaxation delay = 1.7 s, number of scans = 16 and with duration of long-range heteronuclear coupling evolution = 74.4 ms.

In our last example we demonstrate the utility of our method for the measurement of long-range ^{1}H-^{13}C coupling constants in a simple monosaccharide derivative (**V**) (Figure 6, see structure with numbering of atoms in the inset). It is important to note here that, because of the significant sensitivity drop caused by the slice-selective proton pulse and the unfavorable abundance of the carbon-13 nucleus, this experiment works only with highly concentrated (~ M range) samples. However, useful ideas which have recently been reported can significantly improve the sensitivity of Zangger-Sterk-type experiments. For example, with the use of multiple-frequency shaped pulses[51] or changing the offset of the selective shaped pulse after each scan[52] and/or using advanced cryo-probes, the sensitivity of the broadband proton-proton - decoupled CPMG-HSQMBC experiment can be considerably enhanced. With these advances the proposed method should become suitable for the determination of $^{n}J(^{1}H, ^{13}C)$ values under more realistic sample conditions.

Conclusions

A Zangger-Sterk based broadband proton-proton - decoupled CPMG-HSQMBC method has been devised for the precise and direct measurement of multiple bond heteronuclear coupling constants. In the experiment proposed the undesired proton-proton splittings are removed from the heteronuclear multiplets, thus the long-range heteronuclear couplings of interest can be determined from the resulting spectra simply by measuring the frequency differences between the peak maxima of pure antiphase doublets. However, when the coupling constant of interest is comparable to the proton linewidth, direct analysis of the antiphase signal can lead to overestimation of the magnitude of coupling. In such cases, separate recording of complementary in-phase (IP) data with a modified decoupled CPMG-HSQMBC sequence, including an additional refocusing period, allows editing the α/β multiplet components according to the well-known IPAP approach.[53] The potential of the method has been demonstrated on molecules possessing extended networks of mutually coupled protons. For such cases additional multiplet fitting procedures would normally be required to extract long-range heteronuclear couplings from the complex signal patterns obtained when using standard HSQMBC experiments. It has been also illustrated that our novel method allows the measurement of a wide range of multiple bond heteronuclear coupling constants in a single experiment. It is important to note that with the use of multiple-frequency shaped pulses and/or sensitive cryo-probes, the determination of heteronuclear long-range couplings for low-abundance nuclei may become feasible even for samples of modest concentration. Further improvement in sensitivity can be expected from incorporation of the recent PSYCHE[42] pulse sequence element or of instant (real-time) homonuclear broadband decoupling[30] methodology. Implementation of these approaches in the CPMG-HSQMBC sequence is under investigation by our group.

Experimental Section

All experiments were performed on a Bruker Avance II 500 spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) equipped with a BBI or a TXI z-gradient probe. All spectra were processed with TopSpin 2.1, 2.5 or 3.0 (Bruker Biospin GmbH, Karlsruhe, Germany). For testing the broadband proton-proton - decoupled pseudo 1D CPMG-HSQMBC experiment, a sample of 100 mg 1,2:3,4-Di-*O*-isopropylidene-6-seleno-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-galactopyranose (**I**) dissolved in 500 µl $CDCl_3$, a sample of 20.1 mg sodium salt of uridine 2',3'-cyclic monophosphate (cUMP.Na) (**II**) dissolved in 500 µl D_2O, and a sample of 30 mg *cis*-[bis(1-(1-naphthyl)-3-methyl-3-phospholeno)-dichloro-platinum(II)] (**III**) dissolved in 500 µl $CDCl_3$ were used. The broadband proton-proton - decoupled pseudo 2D CPMG-HSQMBC spectra were acquired on a sample of 117.5 mg Methyl 2,3-di-*O*-acetyl-4-seleno-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-seleno)-6-*O*-benzyl-α-D-galactopyranoside (**IV**) dissolved in 550 µl C_6D_6 and on a sample of 320 mg Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**V**) dissolved in 700 µl DMSO-d_6. For all measurements the nominal temperature was set to 298 K unless indicated otherwise.

In order to provide simultaneous composite π pulses on the ^{1}H and the X channel, power levels were carefully calibrated to give equal durations for proton and heteronucleus pulses. Spectra of selenium-containing compounds (Figures 2 and 5) were recorded with proton and selenium 90° pulses of 15 µs. Spectra of phosphorus-containing compounds (Figures 3 and 4) were collected with proton and phosphorus 90° pulses of 16 µs. The broadband proton-proton - decoupled ^{1}H-^{13}C CPMG-HSQMBC spectrum (Figure 6) was acquired with proton and carbon 90° pulses of 16 µs. However, when temperature-sensitive nuclei are being measured and/or a cryogenic probe is used, a CPMG cycle at reduced power level[18] (to give a 90° pulse of ca. 30 µs) is recommended to minimize the heating of the sample and/or to protect probe electronics. Also, if compatible with the proton spectral parameters, the interpulse delays within the CPMG block can be increased (up to ca. 200-250 µs), for the same purpose.

For processing the 2D and 3D raw data sets acquired with the pulse sequence presented, a Bruker AU program (available at http://nmr.chemistry.manchester.ac.uk) was used to reconstruct the 1D and 2D interferograms. The pseudo 1D data were multiplied with a shifted sine-squared function, zero-filled to 16k and then Fourier transformed to yield a spectral resolution of 0.1 – 0.3 Hz/point in the ^{1}H dimension. Prior to 2D Fourier transformation the pseudo 2D data were multiplied with a shifted sine-squared function, zero-filled to 8k in the ^{1}H dimension and multiplied with a shifted sine-squared function, and zero-filled to 256 (Figure 5) and 512 (Figure 6) in the X dimension, before transformation to yield a spectral resolution of 0.2 – 0.4 Hz/point in the ^{1}H dimension.

Other experimental details are given in the figure legends.

Acknowledgements

The authors thank Sára Balla for her skilful technical assistance in preparation of samples. Dr. Lajos Kovács, Prof. György Keglevich and Péter Bagi are acknowledged for their generous gifts of nucleotides and the Pt-complex sample, respectively. Financial support from TÁMOP-4.2.2/A-11/1/KONV-2012-0025, OTKA K 105459 and OTKA NN 109671 (to K.E.K and L.Sz.), from Richter Gedeon Talentum Alapítvány (Ph.D. scholarship to I.T.), from the Engineering and Physical Sciences Research Council (grant numbers EP/I007989 and EP/L018500) (to R.W.A., M.N. and G.A.M.) is gratefully acknowledged. The research of István Timári was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4. A/2-11-1-2012-0001 'National Excellence Program'.

Keywords: broadband proton-proton decoupling • HSQMBC • long-range heteronuclear couplings • NMR spectroscopy • structure elucidation

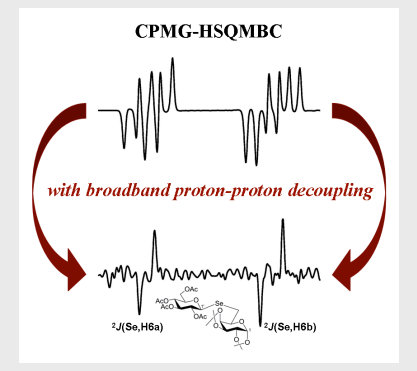
[1] R. T. Williamson, A. V. Buevich, G. E. Martin, T. Parella, *J. Org. Chem.* **2014**, *79*, 3887-3894.
[2] N. Matsumori, D. Kaneno, M. Murata, H. Nakamura, K. Tachibana, *J. Org. Chem.* **1999**, *64*, 866-876.
[3] G. Bifulco, P. Dambruoso, L. Gomez-Paloma, R. Riccio, *Chem. Rev.* **2007**, *107*, 3744-3779.
[4] B. L. Marquez, W. H. Gerwick, R. T. Williamson, *Magn. Reson. Chem.* **2001**, *39*, 499-530.
[5] T. Parella, J. F. Espinosa, *Prog. Nucl. Magn. Reson. Spectrosc.* **2013**, *73*, 17-55.
[6] M. Kurz, P. Schmieder, H. Kessler, *Angew. Chem.* **1991**, *103*, 1341-1342.; *Angew. Chem. Int. Ed.* **1991**, *30*, 1329-1331.
[7] D. Uhrin, G. Batta, V. J. Hruby, P. N. Barlow, K. E. Kövér, *J. Magn. Reson.* **1998**, *130*, 155-161.
[8] K. E. Kövér, V. J. Hruby, D. Uhrín, *J. Magn. Reson.* **1997**, *129*, 125-129.
[9] W. Koźmiński, *J. Magn. Reson.* **1999**, *137*, 408-412.
[10] P. Nolis, T. Parella, *J. Magn. Reson.* **2005**, *176*, 15-26.
[11] K. Kobzar, B. Luy, *J. Magn. Reson.* **2007**, *186*, 131-141.
[12] A. Bax, M. F. Summers, *J. Am. Chem. Soc.* **1986**, *108*, 2093-2094.
[13] K. Furihata, H. Seto, *Tetrahedron Lett.* **1999**, *40*, 6271-6275.
[14] K. Furihata, M. Tashiro, H. Seto, *Magn. Reson. Chem.* **2010**, *48*, 179-183.
[15] R. T. Williamson, B. L. Marquez, W. H. Gerwick, K. E. Kövér, *Magn. Reson. Chem.* **2000**, *38*, 265-273.
[16] H. Koskela, I. Kilpelainen, S. Heikkinen, *J. Magn. Reson.* **2003**, *164*, 228-232.
[17] K. E. Kövér, G. Batta, K. Fehér, *J. Magn. Reson.* **2006**, *181*, 89-97.
[18] S. Boros, K. E. Kövér, *Magn. Reson. Chem.* **2011**, *49*, 106-110.
[19] S. Gil, J. F. Espinosa, T. Parella, *J. Magn. Reson.* **2011**, *213*, 145-150.
[20] J. Saurí, J. F. Espinosa, T. Parella, *Angew. Chem.* **2012**, *124*, 3985-3988.; *Angew. Chem. Int. Ed.* **2012**, *51*, 3919-3922.
[21] B. Baishya, C. L. Khetrapal, *J. Magn. Reson.* **2014**, *242*, 143-154.
[22] L. Castañar, J. Saurí, P. Nolis, A. Virgili, T. Parella, *J. Magn. Reson.* **2014**, *238*, 63-69.
[23] J. A. Aguilar, G. A. Morris, A. M. Kenwright, *RSC Adv.* **2014**, *4*, 8278-8282.
[24] S. R. Chaudhari, N. Suryaprakash, *RSC Adv.* **2014**, *4*, 15018-15021.
[25] K. Zangger, H. Sterk, *J. Magn. Reson.* **1997**, *124*, 486-489.
[26] M. Nilsson, G. A. Morris, *Chem. Commun.* **2007**, 933-935.
[27] J. A. Aguilar, S. Faulkner, M. Nilsson, G. A. Morris, *Angew. Chem.* **2010**, *122*, 3993-3995.; *Angew. Chem. Int. Ed.* **2010**, *49*, 3901-3903.
[28] G. A. Morris, J. A. Aguilar, R. Evans, S. Haiber, M. Nilsson, *J. Am. Chem. Soc.* **2010**, *132*, 12770-12772.
[29] J. A. Aguilar, A. A. Colbourne, J. Cassani, M. Nilsson, G. A. Morris, *Angew. Chem.* **2012**, *124*, 6566-6569.; *Angew. Chem. Int. Ed.* **2012**, *51*, 6460-6463.
[30] N. H. Meyer, K. Zangger, *Angew. Chem.* **2013**, *125*, 7283-7286; *Angew. Chem. Int. Ed.* **2013**, *52*, 7143-7146.
[31] N. Helge Meyer, K. Zangger, *Chem. Commun.* **2014**, *50*, 1488-1490.
[32] P. Sakhaii, B. Haase, W. Bermel, *J. Magn. Reson.* **2009**, *199*, 192-198.
[33] J. A. Aguilar, M. Nilsson, G. A. Morris, *Angew. Chem.* **2011**, *123*, 9890-9891; *Angew. Chem. Int. Ed.* **2011**, *50*, 9716-9717.
[34] A. Lupulescu, G. L. Olsen, L. Frydman, *J. Magn. Reson.* **2012**, *218*, 141-146.
[35] L. Paudel, R. W. Adams, P. Király, J. A. Aguilar, M. Foroozandeh, M. J. Cliff, M. Nilsson, P. Sándor, J. P. Waltho, G. A. Morris, *Angew. Chem.* **2013**, *125*, 11830-11833.; *Angew. Chem. Int. Ed.* **2013**, *52*, 11616-11619.
[36] T. Reinsperger, B. Luy, *J. Magn. Reson.* **2014**, *239*, 110-120.
[37] I. Timári, L. Kaltschnee, A. Kolmer, R. W. Adams, M. Nilsson, C. M. Thiele, G. A. Morris, K. E. Kövér, *J. Magn. Reson.* **2014**, *239*, 130-138.
[38] A. J. Pell, R. A. E. Edden, J. Keeler, *Magn. Reson. Chem.* **2007**, *45*, 296-316.
[39] L. Castañar, P. Nolis, A. Virgili, T. Parella, *Chem. Eur. J.* **2013**, *19*, 17283-17286.
[40] P. Sakhaii, B. Haase, W. Bermel, *J. Magn. Reson.* **2013**, *228*, 125-129.
[41] R. W. Adams, L. Byrne, P. Király, M. Foroozandeh, L. Paudel, M. Nilsson, J. Clayden, G. A. Morris, *Chem. Commun.* **2014**, *50*, 2512-2514.
[42] M. Foroozandeh, R. W. Adams, N. J. Meharry, D. Jeannerat, M. Nilsson, G. A. Morris, *Angew. Chem.* **2014**, *126*, 7110-7112.; *Angew. Chem. Int. Ed.* **2014**, *53*, 6990-6992.
[43] M. Foroozandeh, R. W. Adams, M. Nilsson, G. A. Morris, *J. Am. Chem. Soc.* **2014**, *136*, 11867-11869.
[44] J. Ying, J. Roche, A. Bax, *J. Magn. Reson.* **2014**, *241*, 97-102.
[45] J. R. Garbow, D. P. Weitekamp, A. Pines, *Chem. Phys. Lett.* **1982**, *93*, 504-509.
[46] N. H. Meyer, K. Zangger, *ChemPhysChem* **2014**, *15*, 49-55.
[47] E. Kupce, J. Boyd, I. D. Campbell, *J. Magn. Reson., Ser B* **1995**, *106*, 300-303.
[48] K. E. Kövér, A. A. Kumar, Y. Y. Rusakov, L. B. Krivdin, T. Z. Illyés, L. Szilágyi, *Magn. Reson. Chem.* **2011**, *49*, 190-194.
[49] Y. Y. Rusakov, L. B. Krivdin, A. A. Kumar, L. Szilágyi, K. E. Kövér, *Magn. Reson. Chem.* **2012**, *50*, 488-495.
[50] P. Bagi, T. Szilvási, P. Pongrácz, L. Kollár, L. Drahos, G. Keglevich, *Curr. Org. Chem.* **2014**, *18*, 1529-1538.
[51] L. Castañar, P. Nolis, A. Virgili, T. Parella, *Chem. Eur. J.* **2013**, *19*, 15472-15475.
[52] P. Sakhaii, B. Haase, W. Bermel, R. Kerssebaum, G. E. Wagner, K. Zangger, *J. Magn. Reson.* **2013**, *233*, 92-95.
[53] S. Gil, J. F. Espinosa, T. Parella, *J. Magn. Reson.* **2010**, *207*, 312-321.

Entry for the Table of Contents (Please choose one layout)

Layout 1:

FULL PAPER

A broadband proton-proton - decoupled CPMG-HSQMBC method is proposed for the precise and direct measurement of long-range heteronuclear coupling constants. The broadband homodecoupling scheme incorporated in the experiment efficiently eliminates unwanted proton-proton splittings from the heteronuclear multiplets, allowing the heteronuclear couplings of interest to be determined simply by measuring frequency differences between singlet maxima of pure antiphase doublets.



*István Timári, Tünde Z. Illyés, Ralph W. Adams, Mathias Nilsson, László Szilágyi, Gareth A. Morris, and Katalin E. Kövér**

Page No. – Page No.

Precise measurement of long-range heteronuclear coupling constants by a novel broadband proton-proton - decoupled CPMG-HSQMBC method