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A New Two-Dimensional Pulse Sequence for T_2^* Measurements of Protons in ^{13}C Isotopomers

Jörg Lambert,[1] Stephan Haiber, and Helmut Herzog

Institut für Spektrochemie und Angewandte Spektroskopie, P.O. Box 10 13 52, D-44013 Dortmund, Germany

E-mail: lambert@isas-dortmund.de

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A new two-dimensional pulse sequence for T_2^* measurement of protons directly coupled to ^{13}C spins is proposed. The sequence measures the tranverse relaxation time of heteronuclear proton single-quantum coherence under conditions of free precession and is therefore well suited to evaluate relaxation losses of proton magnetization during preparation delays of heteronuclear pulse experiments in analytical NMR. The relevant part of the pulse sequence can be inserted as a "building block" into any direct or inverse detecting H,C correlation pulse sequence if proton spin–spin relaxation is to be investigated. In this contribution, the building block is inserted into a HETCOR as well as into a HMQC pulse sequence. Experimental results for the HETCOR-based sequence are given.

Key Words: **relaxation time; T_2^* measurement; BIRD pulse; ^{13}C isotopomers; heteronuclear shift correlation.**

INTRODUCTION

NMR is a valuable tool for the quantitative analysis of mixtures of organic compounds covering a wide range of molecular sizes such as humic substances (*1–3*) or mineral oils (*4*). In these systems, NMR delivers results unobtainable by other spectroscopic or chemical techniques. The most valuable pulse sequences for organic mixture analysis such as HETCOR, HMQC (*5*), and HSQC (*6*) employ polarization transfer with preparation delay times in the range of several milliseconds and are thus susceptible to quantification errors if proton spin–spin relaxation times are of the order of milliseconds. A reliable estimation of the quantification errors when integrating signals or crosspeaks therefore requires the knowledge of spin–spin relaxation times of protons of ^{13}C-isotopomers. T_2^* times for protons of ^{13}C isotopomers are substantially smaller than those of ^{12}C isotopomers due to an additional contribution from ^{13}C, ^{1}H dipole–dipole relaxation. The latter are therefore not suitable for evaluating quantification losses. Whereas relaxation times of protons coupled to ^{12}C are easily accessible by the CPMG (*7*, *8*) technique, the measurement of T_2^* times of protons coupled to ^{13}C requires special techniques.

[1] To whom correspondence should be addressed.

If relaxation times for several proton sites are to be obtained, two-dimensional NMR is more convenient than 1D techniques, as assignment and ambiguity problems are avoided. We here propose a new two-dimensional pulse technique for spin–spin relaxation time measurement of protons coupled to ^{13}C nuclei. The relevant pulse sequence element that is incremented for T_2^* relaxation measurements can be implemented as a "building block" into direct detection C,H correlation techniques like HETCOR as well as into inverse H,C correlation pulse techniques like HMQC or HSQC.

RESULTS

The two-dimensional measurement of T_2^* relaxation times for proton spins directly bound to ^{13}C requires a dedicated delay (relaxation delay τ_R) to be inserted into a C,H correlation pulse sequence. During this delay time, merely spin–spin relaxation is supposed to act on the in-phase proton magnetization. Modulation due to proton chemical shift and proton–proton and carbon–proton coupling must not occur. These three relevant interactions acting on proton magnetization are therefore to be refocused during the delay time τ_R. In the case of τ_R being as long as several hundred milliseconds, the evolution of the proton magnetization under proton–proton couplings is to be considered as well.

Refocusing of the proton chemical shift and the couplings between protons bound to ^{12}C nuclei and protons bound to ^{13}C nuclei is accomplished by insertion of a bilinear π pulse (*9*) into the center of the relaxation delay time τ_R. In Figs. 1 and 2 the BIRD cluster is shown in the center of the relaxation delay time τ_R (illustrated in grayscale) of the displayed pulse sequences (HETCOR in Fig. 1 and HMQC in Fig. 2). The BIRD π pulse with all phases of proton pulses being $+y$ acts on the protons bound to ^{13}C nuclei, whereas protons bound to ^{12}C experience an identity transform, resulting in refocusing of the J(H,H) coupling. ^2J(H,H) couplings of CH_2 groups with chemically nonequivalent protons, however, are not refocused, as both of these protons are coupled to ^{13}C and experience π pulses. As those CH_2 groups are easily identified in C,H correlation

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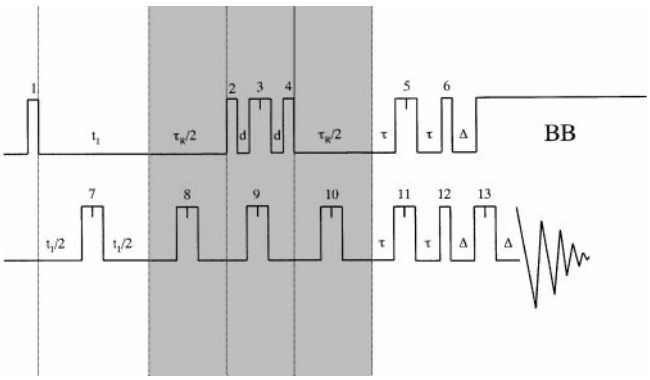

FIG. 1. Pulse sequence for T_2^* relaxation time measurements of proton spins coupled to ^{13}C nuclei, based on the phase-sensitive HETCOR experiment. Pulses without and with vertical bars denote $\pi/2$ and π pulses, respectively. The "building block" used for relaxation time measurement is illustrated in grayscale. τ_R denotes the freely adjustable delay time for proton T_2^* relaxation. The delay times τ and Δ are set to $1/4J$(C,H) each. The delay times d of the BIRD sequence are adjusted according to $1/2J$(C,H) each. The phase ϕ_1 is $-y, +y, -y, +y$ for the real States (*10*) data and $+x, -x, +x, -x$ for the imaginary States data. Phase ϕ_5 and phases ϕ_7–ϕ_{13} are $+x$ throughout; phases ϕ_2–ϕ_4 are $+y$ throughout. Phase ϕ_6 is $+x, +x, -x, -x$; ϕ_{rec} is $+x, -x, -x, +x$. The proton broadband decoupling starts at the beginning of the second Δ delay to avoid an additional proton π pulse after the first Δ delay.

experiments, this shortcoming of the proposed technique does not impose any real problems.

For the one-bond carbon–proton couplings, however, the BIRD puls acts on both the ^{13}C nucleus and the proton bound to the ^{13}C nucleus, causing the ^1J(C,H) to diverge during the relaxation delay time τ_R. For this reason we inserted two further ^{13}C π pulses, one in the center of the first half and one in the center of the second half of the relaxation delay time (Fig. 1). Each of these π pulses serves to refocus the C,H coupling during the first and the second halves of the relaxation delay time τ_R. Hence, the BIRD pulse acts on proton magnetization that is in phase with respect to the one-bond J(C,H) coupling.

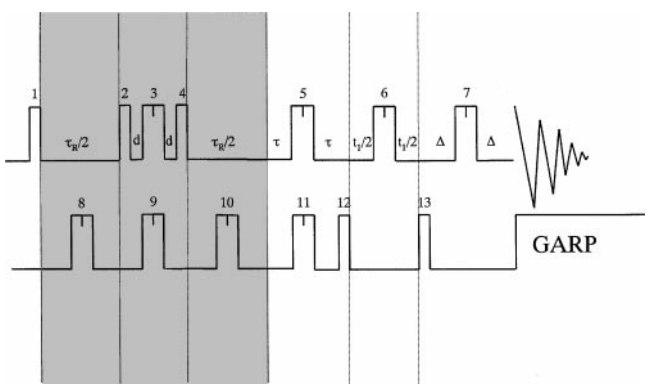

FIG. 2. Pulse sequence for T_2^* relaxation time measurements of proton spins coupled to ^{13}C nuclei, based on the phase-sensitive HMQC experiment. Pulses without and with vertical bars denote $\pi/2$ and π pulses, respectively. The "building block" used for relaxation time measurement is given in grayscale. τ_R denotes the freely adjustable delay time for proton T_2^* relaxation. The delay times τ and Δ are set to $1/4J$(C,H) each. The delay times d of the BIRD sequence are adjusted according to $1/2J$(C,H) each.The phases ϕ_1 and ϕ_5–ϕ_{11} are $+x$ throughout; phases ϕ_2–ϕ_4 are $+y$ throughout. Phase ϕ_{12} is $+x, +x, -x, -x$; ϕ_{13} is $+x$ for the real States data and $+y$ for the imaginary States (*10*) data; ϕ_{rec} is $+x, +x, -x, -x$.

EXPERIMENTAL

In this contribution, we use the carbon-detected HETCOR experiment to demonstrate the performance of the proposed refocusing scheme, as no measures have to be taken to remove the strong and impeding signal of HDO or H_2O from the spectrum. The suggested building block is inserted into the standard phase-sensitive HETCOR experiment in between the t_1-labeling period and the period for polarization transfer (Fig. 1). The proton broadband decoupling starts at the beginning of the second Δ delay to avoid an additional proton π pulse after the first Δ delay.

The spectra were measured on a Jeol GX400 NMR spectrometer operating at 400 and 100 MHz for ^{1}H and ^{13}C, respectively. The delay times τ and Δ of the pulse sequence (Fig. 1) are adjusted to the one-bond ^{13}C,^{1}H coupling constant ^1J(C,H) according to the relationship $\tau = \Delta = 1/(4 \cdot {}^1J(\text{C,H}))$. A ^1J(C,H) value of 140 Hz was used for all measurements. A total of 128 t_1 increments were used for the 2D experiments, and frequency ranges in the second and the first dimension were 2.8 and 12 kHz, respectively. Phase-sensitive data were calculated according to the method of States *et al.* (*10*).

The signal-to-noise ratio (S/N) of the crosspeaks was in the range of 30–50, depending on the value of τ_R. Volume integration of the crosspeaks was performed using Jeol Delta software, version 3.0. The uncertainties of the individual data points range from 3 to 5% depending on the S/N of the integrated crosspeaks.

DISCUSSION AND CONCLUSIONS

The sequence of Fig. 1 was checked with a sample of 85 mg of L-rhamnose in D_2O. A set of 2D spectra applying the pulse sequence of Fig. 1 was taken with seven delay times τ_R varying from 100 to 700 ms. The crosspeaks of the C_5/H_5 connectivity as well as that of the methyl group of L-rhamnose were integrated and the resulting volume integrals were fitted to a decaying exponential function. Proton T_2^* times of 459 ± 23 and 692 ± 35 ms for the CH_3 group protons and the H_5 proton, respectively, were found (Fig. 3). Carr–Purcell–Meiboom–Gill (*7, 8*) measurements deliver 508 ± 15 and 751 ± 23 ms for the proton H_5 and the CH_3 proton, respectively, reflecting the effect of the missing ^{13}C, ^{1}H dipole–dipole relaxation pathway.

The sequence measures the tranverse relaxation time of heteronuclear proton single-quantum coherence under conditions of free precession and is therefore well suited to evaluate relaxation losses of proton magnetization during preparation delays

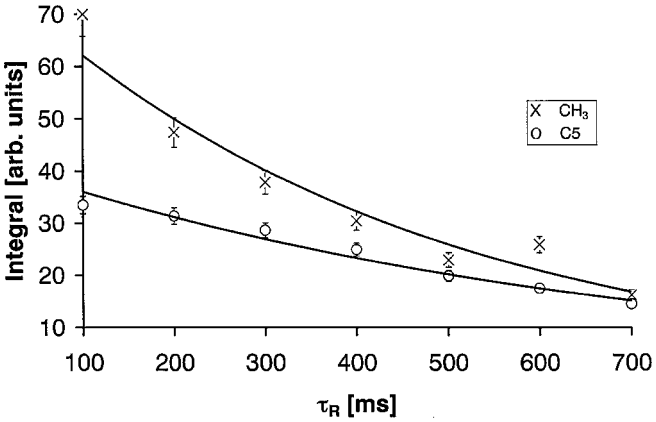

FIG. 3. T_2^* decay curves for the volume integrals of the C_5/H_5 crosspeak and the CH_3 crosspeak of L-rhamnose, obtained with the pulse sequence of Fig. 1. The uncertainties for the individual data points range from 3 to 5% depending on the S/N of the integrated crosspeaks. The curve fit of the volume integrals to an exponential function delivered proton T_2^* times of 459 ± 23 and 692 ± 35 ms for the CH_3 group protons and the H_5 proton, respectively.

of heteronuclear pulse experiments in analytical NMR. Due to the inclusion of two carbon π pulses in the τ_R delay, the pulse sequence suppresses contributions originating from cross correlation between the proton chemical shift anisotropy relaxation mechanism (CSA (^{1}H)) and the ^{13}C, ^{1}H dipolar relaxation mechanism (^{13}C, ^{1}H-DD). For the range of spin–spin relaxation times relevant in analytical applications of NMR, contributions from CSA(^{1}H)/^{13}C, ^{1}H dipolar cross correlation should be negligible, as the relaxation times are at least 5–10 times larger than the preparation delays of HETCOR or HMQC/HSQC experiments, i.e., the spin flips induced by the ^{13}C π pulse in the center of the preparation delay occur rapidly with respect to the spin–spin relaxation rate (*11*). For spin–spin relaxation times comparable to the length of the preparation delay, cross correlation does contribute to the relaxation of proton magnetization during the preparation delays of HETCOR or HMQC/HSQC experiments: In this case, however, a 2D spectrum can hardly be obtained due to the massive relaxation losses during the preparation delay. To conclude, the new pulse sequence is a valuable tool for measurements of T_2^* relaxation times of proton spins in ^{13}C isotopomers. Nevertheless, the proposed refocusing scheme merely requires proton magnetization to start with and hence can be inserted into any kind of inverse or direct detecting H,C correlation pulse sequence.

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