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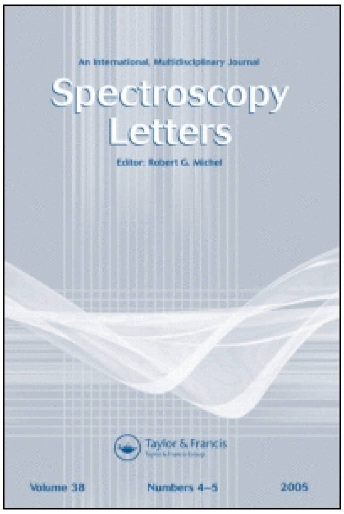

**Spectroscopy Letters**

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

## Fast $^{13}$C-NMR Spectral Editing for Determining CH$_n$ Multiplicities

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**To cite this Article** Bigler, Peter(2008) 'Fast $^{13}$C-NMR Spectral Editing for Determining CH$_n$ Multiplicities', Spectroscopy Letters, 41: 4, 162 — 165

**To link to this Article: DOI:** 10.1080/00387010802008005

**URL:** http://dx.doi.org/10.1080/00387010802008005

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**Spectroscopy Letters**, 41:162–165, 2008
Copyright © Taylor & Francis Group, LLC
ISSN: 0038-7010 print/1532-2289 online
DOI: 10.1080/00387010802008005

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# Fast $^{13}$C-NMR Spectral Editing for Determining $CH_n$ Multiplicities

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**ABSTRACT** With an improved version of the $^{13}$C DEPTQ NMR experiment all carbon multiplicities ($C_q$, CH, $CH_2$ und $CH_3$) can be identified unequivocally and most conveniently in two experiments and at best with only one scan each. This simplifies the analysis of $^{13}$C-NMR data on a routine level in general. With higher sample amounts and/or exploiting the high sensitivity of cryogenically cooled $^{13}$C probeheads the efficiency of such investigations may be improved. This makes this method attractive for fast $^{13}$C analysis of small-to-medium sized molecules in high-throughput laboratories.

**KEYWORDS** $^{13}$C-NMR signal assignment, $^{13}$C-NMR multiplicity determination, DEPTQ, NMR, spectroscopic methods

Multiplicity dependent 1D $^{13}$C-NMR experiments such as DEPT[1] and APT[2] belong to the most common and popular spectroscopic tools for assigning $^{13}$C signals and for the elucidation and characterization of molecular structures on a routine level. When combined with a standard 1D $^{13}$C and a $^{1}$H experiment known molecular structures may be verified and simple structural problems may be solved. Corresponding spectra provide also the basis for planning and setting up advanced NMR experiments in case of more demanding structural problems. According to its low dependence on pulse imperfections and its low J cross-talk dependence on a spread of J couplings DEPT is certainly the most prominent especially for spectral editing, i.e. the calculation of multiplicity selective subspectra. However DEPT yields exclusively the signals of proton bearing carbons $CH_n$, whereas the signals of quaternary carbons $C_q$ are suppressed. Therefore DEPT is combined usually with a standard $^{13}$C one-pulse experiment or a "quaternary-only" experiment[3] for complete multiplicity determination. A DEPT-90 and a DEPT-135 experiment have to be combined with one of these latter experiments for complete and reliable carbon multiplicity determination.

We introduced a modified DEPT experiment DEPTQ[4] yielding the signal and multiplicity information for all carbon types including the signals of quaternary carbons. Compared to the DEPT experiment two further $^{13}$C pulses (90° and 180°) and an extra delay adjusted to $(1/[2\cdot{}^{1}J(CH)])$ had been included at the beginning of the pulse sequence. This additional pulse sequence element has two effects: 1. Quaternary carbons are excited to

Received 27 August 2007;
accepted 19 December 2007.

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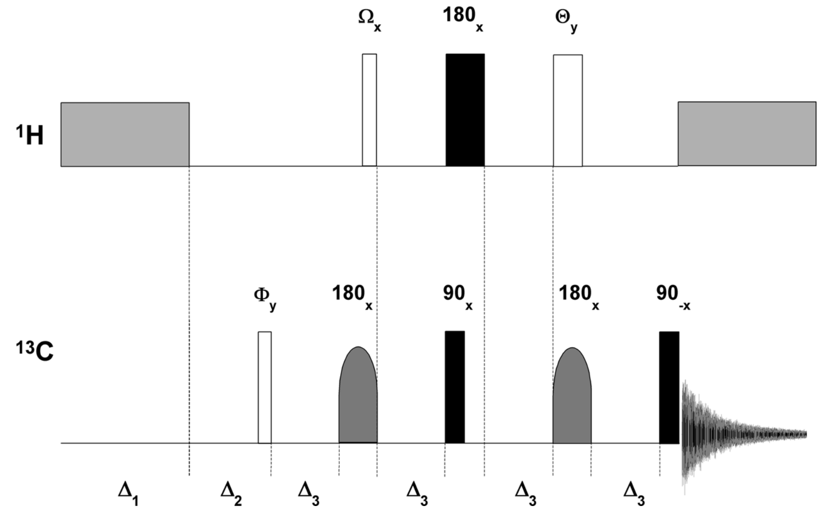
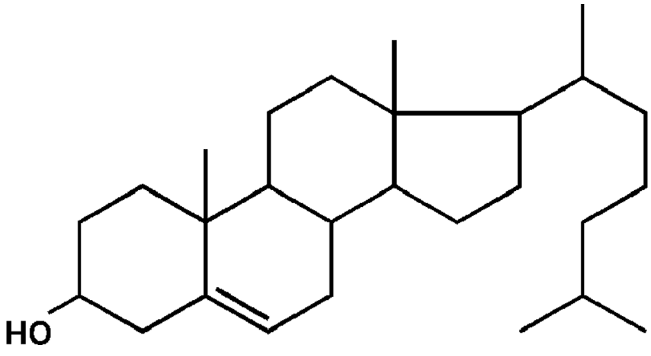
show their signals and the signals of protonated carbons (excited via polarization transfer) with absorptive line shapes throughout. 2. Signals of protonated carbons excited with the initial $^{13}$C pulse are transferred into non-observable zero- and multiquantum coherence with the third (90°) carbon pulse. Consequently and in contrast to the DEPT experiment this pathway does not contribute residual dispersive signals and no phase cycling is therefore necessary to get rid of these unwanted signal components. This allows all carbon multiplicities, including quaternary carbons, to be determined with only two DEPTQ experiments, with the $^{1}$H-"selection pulse" set to 90° and 135° respectively: $C_q$ and CH signals appear with opposite signs in the spectrum of the first experiment, $CH_2/C_q$ and $CH_3$/CH signals appear with opposite signs in the spectrum of the second experiment respectively.

Very recently we proposed a modified version of the DEPTQ experiment (Scheme 1) which has proved to yield $^{13}$C spectra of substantially improved quality compared to its original version.[5]

Signal intensities for the sensitivity limiting quaternary carbons are enhanced by the incorporation of composite adiabatic $^{13}$C refocusing pulses,[6] an adjustable initial carbon pulse (together with an additional 90 carbon pulse prior to data acquisition) and a relaxation period split into two delays with and without $^{1}$H broadband decoupling respectively. The initial $^{13}$C pulse may be adjusted for maximum sensitivity ("Ernst angle") of quaternary carbons exactly in the same way as practiced with the standard $^{13}$C one-pulse experiment. With the two delays $\Delta_1$ and $\Delta_2$ of the split relaxation period the Nuclear Overhauser (NOE) build-up time for the usually weaker quaternary carbons may be prolonged and their intensity further enhanced. This occurs at the cost of a minor decrease for the usually intense signals of protonated carbons. Therefore $\Delta_1$ governing the intensity of quaternary carbons is adjusted first according to the same rules ($\Delta_1 = 1$–$3\ T1^{C}_{max.}$) valid for the relaxation delay in a standard $^{13}$C one-pulse experiment in order to obtain best sensitivity for quaternary carbons. The less critical adjustment of $\Delta_2$ affects the intensity of all types of carbons but should be set as short as possible, typically 0.5–1 s. With $\Delta_2$ set too long the NOE enhancement obtained for the signals of quaternary carbons may be lost, with almost no additional sensitivity gain for the already intense signals of protonated carbons. Exploiting composite adiabatic $^{13}$C refocusing pulses, constructed on the basis of a smoothed chirp pulse and available as standard "accessories" on most modern routine NMR spectrometers, both the sensitivity of the most critical quaternary carbons as well as the line shapes in general may be improved. Pure absorption line



**SCHEME 1** Scheme of the modified DEPTQ pulse sequence. Compared to the first version the initial $^{13}$C and $^{1}$H 90° pulses are replaced by adjustable pulses $\Phi$ and $\Omega$ respectively. They are used to optimize the sensitivity of quaternary carbons ("Ernst angle") and to simplify spectral editing as described in the main text. The $^{13}$C 180° pulses are replaced by composite adiabatic refocusing pulses to suppress "off-resonance effects". The relaxation delay is split into two parts, $\Delta_1$ and $\Delta_2$ respectively. Additional Nuclear Overhauser enhancement for the signals of the usually weaker quaternary carbons occurs in $\Delta_1$ with simultaneous $^{1}$H broadband decoupling, whereas proton relaxation occurs in the shorter $\Delta_2$ delay. $\Theta$ is used as with the DEPT experiment for the differentiation of CH, $CH_2$ and $CH_3$.



**SCHEME 2** Formula of cholesterol.

shapes for all carbon types can be obtained with a single scan, making phase cycling and the use of so-called initial dummy scans superfluous.

Comparative studies with the modified DEPTQ and the standard $^{13}C$ one-pulse experiment applied to various samples reveal an averaged decrease of signal intensities for quaternary carbons of 15–20% compared to the standard $^{13}C$ one-pulse experiment measured under identical conditions (i.e., within the same measuring time). This decrease is believed to originate from relaxation losses in the additional delays of the DEPTQ pulse sequence. Concentrating however on complete carbon multiplicity assignment, it could be shown that an improved overall sensitivity may be achieved within the same total measuring time with DEPTQ-90/DEPTQ-135 compared to a combination of an individually optimized $^{13}C$ one-pulse, a DEPT-90 and a DEPT-135 experiment.

Here a slightly modified final version of DEPTQ with an initial $^{1}H$ pulse of arbitrary length is proposed (Scheme 1), which simplifies spectral editing.

To demonstrate its perfomance the current DEPTQ version has been applied together with a $^{13}C$ one-pulse experiment for comparison to a concentrated sample of cholesterine (Scheme 2) dissolved in $CDCl_3$ as the solvent.

With the value of the first adjustable proton pulse $\Omega$ (or alternatively of the third carbon pulse) set to 45° and 90° for the DEPTQ-90 and DEPTQ-135 experiment respectively the intensities of CH signals in the DEPTQ-90 spectra are reduced to 70% of their

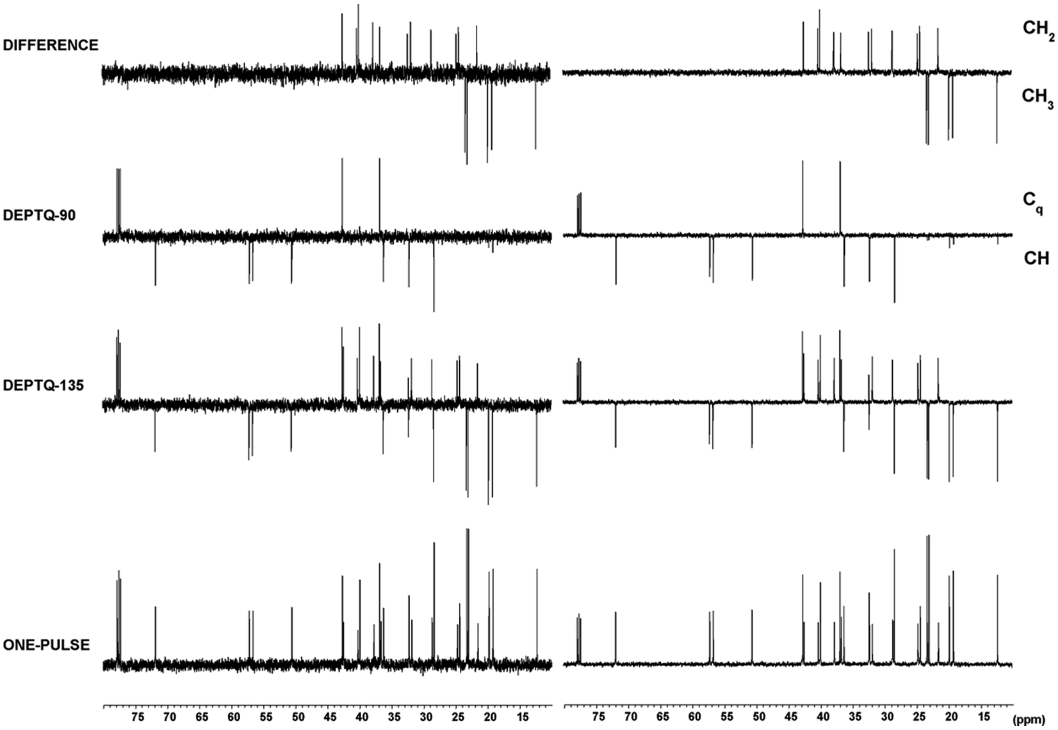


**FIGURE 1** Spectral editing with the improved DEPTQ experiment applied to 350 mg cholesterol dissolved in 750 µl $CDCl_3$. One single scan (left) and eighth scans (right) have been measured with a DEPTQ-90, a DEPTQ-135 and a standard $^{13}C$ one-pulse experiment. $\Omega$ was set to 90° and 45° for the DEPT-135 and DEPTQ-90 experiments and $\Phi$ was adjusted to 90° and 60° in both DEPTQ experiments with the number of scans set one and eighth scans respectively as described in the text. The same lenghts were used for the excitation pulse of the corresponding two one-pulse $^{13}C$ experiments. Expansions of the aliphatic spectral region are shown. $C_q$ and CH carbon multiplicities can be identified in the DEPTQ-90 spectrum; $CH_2$ and $CH_3$ carbon multiplicities can be identified in the difference spectrum (DEPTQ-135–DETPQ-90).

maximum value (with $\Omega = 90°$), i.e., to the value obtained with the DEPTQ-135 experiment. Subtracting these two spectra from each other yields a $CH_2/CH_3$ edited spectrum with the signals of the two multiplicities in opposite phase (Figure 1). The DEPTQ-90 spectrum on the other hand shows exclusively and with opposite phase the signals of $C_q$ and CH. Full spectral editing can therefore be achieved most easily and conveniently with only two experiments and at best with only one scan each.

In case of weak quaternary signals with intensities close to the noise level, their visibility may be improved simply by adding the two spectra obtained with the DEPTQ-90 and DEPTQ-135 respectively.

The experiments DEPTQ-90 ($\Omega$ set to 45°), DEPTQ-135 ($\Omega$ set to 90°) and a $^{13}C$ one-pulse experiment have been applied twice: with the acquisition of one single scan to demonstrate the "one-scan performance" and with eight scans (but no phase cycling) to demonstrate the "editing performance" and the line shape quality of the DEPTQ experiment at an improved signal-to-noise level (Figure 1). To achieve highest $C_q$ signal intensities, the initial carbon pulse $\Phi$ (and the excitation pulse in the corresponding $^{13}C$ one-pulse experiments) has been set to 90° and 60° ("Ernst angle") for the first (single scan) and the second (eight scan) series respectively.

In conclusion a modified DEPTQ experiment has been proposed dedicated for the detection and determination of all types of carbon multiplicities including quaternary carbons. This experiment is most suited for easy and complete spectral editing with a minimum of experiments (two) and a minimum of scans (one) at best. These attributes, and the well-known DEPT inherent advantages such as its robustness with respect to pulse angles and/or $^1J(CH)$ evolution delays deviating from their optimal values, make the improved DEPTQ a valuable alternative for fast $^{13}C$ analysis of small molecules in high throughput laboratories. With high sample amounts and/or the use of cryogenically cooled $^{13}C$ probeheads yielding single scan spectra of adequate quality DEPTQ may become an attractive tool for ultrafast $^{13}C$ analysis.

## EXPERIMENTAL

For this investigation a sample of 350 mg cholesterol was dissolved in 750 µl of CDCl3. The NMR experiments have been performed on a BRUKER DRX500 spectrometer equipped with an ATMA-BBI probehead with $^1H$- and $^{13}C$ 90° pulse lengths of 7.8 µs (−2dB) and 18.5 µs (−3dB) respectively. The sweep width was 149.5 ppm, the number of acquired data points was 64K and the acquisition time was 1.75 s. Two series of experiments (DEPTQ-90, DEPTQ-135 and a standard $^{13}C$ one-pulse experiment) were performed with the number of scans NS set to 1 and 8 respectively. For the $^{13}C$ one-pulse experiment the relaxation delay was set to 31s and 5s with NS = 1 and NS = 8 respectively. For the DEPTQ experiments the two relaxation delays $\Delta_1$, $\Delta_2$ were set to 30s, 1s and 4s, 1s with NS = 1 and NS = 8 respectively. $\Omega$ was adjusted to 90° and 45° for the DEPTQ-135 and DEPTQ-90 experiments. $\Phi$ was adjusted to 90° and 60° in both DEPTQ and the $^{13}C$ one-pulse experiments with NS = 1 and 8 respectively. The pulse sequence for BRUKER spectrometers is available on request.

## ACKNOWLEDGEMENT

I acknowledge helpful discussions with Dr. Wolfgang Bermel, BRUKER BioSpin GmbH, Am Silberstreifen 4, D-76287 Rheinstetten and Rainer Kümmerle, BRUKER BioSpin AG, Industriestrasse 26, CH-8117 Fällanden.

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