ORGANIC LETTERS

2012
Vol. 14, No. 2
524–527

Application of the PROJECT Concept for Suppression of *J* Modulation to DEPT for ^{13}C-Multilabeled Analytes

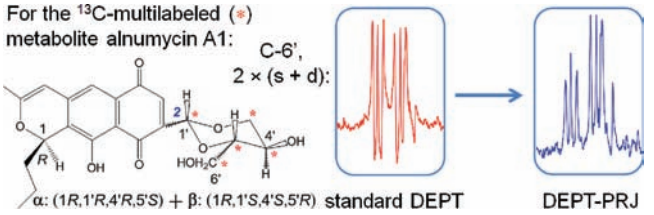
Karel D. Klika*

Department of Chemistry, University of Turku, Vatselankatu 8, FIN-20014 Turku, Finland

klikakd@yahoo.co.uk

Received November 23, 2011

ABSTRACT



Incorporation of the PROJECT element for suppression of *J* modulation into the DEPT pulse sequence resulted in near-distortionless signals, thus realizing spectra more amenable to quantitative evaluation, a potential valuable aid in cases where ^{13}C-multilabeled compounds arise, e.g. as a result of feeding experiments in biosynthetic studies.

For pulse sequences that incorporate a spin echo (e.g., a 180° pulse for refocusing, a ubiquitous feature of many pulse sequences), an undesirable consequence is that while the chemical shift is refocused, the homonuclear scalar coupling (J) is not.[1] The resulting perturbation of the observed signals is referred to as J modulation. The manner in which J modulation manifests itself will be dependent on various factors such as the magnitude of J and the spin−echo time, and so the signal perturbation will not always be clearly comprehensible. Very recently, Morris et al.[1] presented a succinct account of a pulse sequence element, denoted as 'PROJECT' (Periodic Refocusing Of J Evolution by Coherence Transfer) that suppresses homonuclear-induced J modulation and which is apparently general in its application (supported by theoretical treatment) as well as elegant in its simplicity. The method, consisting simply of a double spin echo of two 180° pulses interspaced by a 90° pulse, is actually not new,[2] but its potential application has evidently not been previously appreciated let alone realized. Morris et al.,[1] as well as presenting an excellent example of a T_2 application by incorporation of the PROJECT element into the Carr−Purcell−Meiboom−Gill[3] (CPMG) pulse sequence, allude to the potential applications of the PROJECT principle.

One application that immediately comes to the fore in terms of benefit arises in biosynthetic studies that utilize the uptake of ^{13}C-multilabeled substrates followed by NMR detection of the metabolites. DEPT is preferable to Cr(acac)$_3$ addition and normal ^{13}C acquisition because (1) adulteration of the sample may not be desired; (2) in any case, the relaxation of ^{1}H nuclei will still be faster than ^{13}C nuclei in a sample with added Cr(acac)$_3$ and thus faster repetition rates can be utilized since the regeneration of detected magnetization in the experiment relies on the faster relaxing ^{1}H nuclei from whence it originates; and finally, (3) sensitivity can be significantly degraded due to increased line width and loss of NOE.

Two problems with DEPT, however, are that the response is dependent on the value of J for which the acquisition is optimized and the aforementioned J modulation in the case of ^{13}C-multilabeled analytes. The former limitation can be circumvented by use of a control

(1) Aguilar, J. A.; Nilsson, M.; Bodenhausen, G.; Morris, G. A. *Chem. Commun.* **2012**, *48*, 811.

(2) (a) Takegoshi, K.; Ogura, K.; Hikichi, K. *J. Magn. Reson.* **1989**, *84*, 611. (b) van Zijl, P. C.; Moonen, C. T. W.; von Kienlin, M. J. *J. Magn. Reson.* **1990**, *89*, 28. (c) Torres, A. M.; Zheng, G.; Price, W. S. *Magn. Reson. Chem.* **2010**, *48*, 129.

(3) (a) Carr, H. Y.; Purcell, E. M. *Phys. Rev.* **1954**, *94*, 630. (b) Meiboom, S.; Gill, D. *Rev. Sci. Instrum.* **1958**, *29*, 688.

10.1021/ol2031334
Published on Web 12/30/2011

sample and each multiplet in the analytical sample is then evaluated with respect to their corresponding signal in the control, both sets standardized to an internal standard. This approach, though robust, has reliability limits for small samples composed of natural abundance isotopomers. Alternatively, the *J*-dependent response can be rendered effectively invariant over a prescribed range of *J* values by application of the QHSQC/ACCORD principle[4] by cycling the interpulse delays and the ^{1}H-selection pulse.

However, because of *J* modulation, not only is the quantitation compromised, but the severe distortion that can occur for multiplets can render them problematic for interpretation, or even make them practically incomprehensible altogether in cases where several isotopomers are present. Restoration of normal line intensities can thus alleviate the interpretative difficulties in such cases. Herein we report on the incorporation of the pulse sequence element PROJECT into the DEPT[5] experiment, hereafter denoted as DEPT-PRJ and depicted in Figure 1.

Prior to commencement of the work and modification of the DEPT pulse sequence, the required pulses for the experiment were duly calibrated.[6] For development of the pulse sequence, a sample of fully ^{13}C-labeled D-glucose ([6-$^{13}C_{1-6}$]-D-glucose) in D_2O was utilized. Starting from the standard DEPT pulse sequence, it was realized that a pulse train consisting of 90°−Δ−180°−Δ could be initiated immediately at the end of the DEPT pulse sequence to imitate the PROJECT spin echo since one spin echo is already part of the end of the DEPT pulse sequence itself. For the suppression of *J* modulation to be effected, the coupled ^{13}C spins must all share a concurrent state,[1] and this is indeed the case as the ^{13}C magnetization is in phase after the spin echo of the DEPT pulse sequence following which the newly introduced 90° pulse elicits coherence transfer. To dispense with the evolution arising from coupling to ^{1}H nuclei, decoupling was also initiated at this time so that only homonuclear $J_{C,C}$ was in effect. It was unnecessary to set the delay times (Δ_2) within the PROJECT spin echo to $1/(2 \times J_{H,C})$ as per normal DEPT acquisition, and they were set as an independent variable with typical values lying in the range 1−3 ms. The phases of the pulses within the PROJECT spin echo simply followed that of the 180° ^{13}C-pulse in the DEPT pulse sequence proper, and moreover, it was it was found necessary to utilize a full phase cycle (32 transients) as implemented in the standard DEPT experiment.

Since Morris et al.[1] ascribe beneficial effects from multiple spin echoes, this option was implemented, and consequently it was found that multiple spin echoes could provide further improvements. Finally, to address the problem of when a range of $J_{H,C}$ values are present, the QHSQC principle incorporating variable times for the interpulse delays within the DEPT part of the experiment (Δ_1) and variable flip angles for the ^{1}H-selection pulse to render results independent of *J* over a prescribed range[4d] was also implemented. With a total of eight delay times and six flip angles, together with a full phase cycle of 32, this led to a sizable quotient of scans ($8 \times 6 \times 32 = 1.5$k) for this option, but this was considered inconsequential given that acquisitions for real samples would normally involve substantial temporal accumulations.[7a]

The modified pulse sequences, DEPT-PRJ in its basic form together with optional variants for multiple spin echoes (indicated by an appended "MULT") and/or application of the QHSQC principle (indicated by a prepended "Q"), thus four sequences in total, are depicted in Figure 1. An acquisition using the DEPT-PRJ.MULT pulse sequence yielding near-perfect in-phase spectra, with comparison to a normal DEPT acquisition for which the multiplets are hopelessly distorted, for the setup sample of [6-$^{13}C_{1-6}$]-D-glucose is portrayed in Figure 2 with the conditions applied described in the figure caption.

Adiabatic pulses were found to have a detrimental effect on the spectra after implementation, and their incorporation were not persevered with; also, successful parametri-

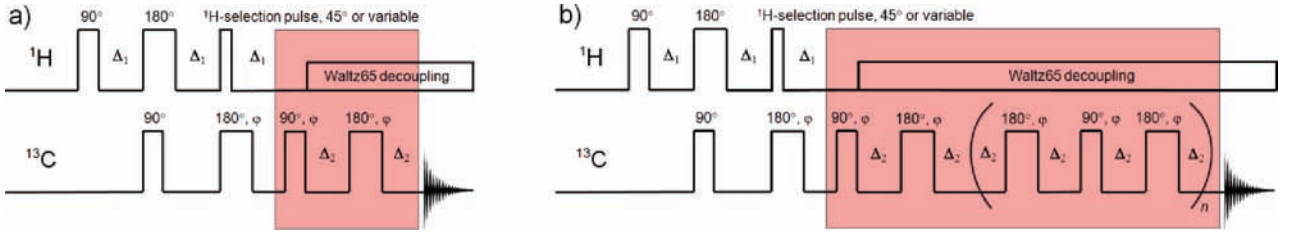


Figure 1. Schematic of the DEPT-PRJ pulse sequences with the additional segments comprising the PROJECT element shaded. In (a) is depicted the basic DEPT-PRJ pulse sequence wherein $\Delta_1 = 1/(2 \times J_{H,C})$ as in normal DEPT acquisition or is cycled as per Jiang et al.[4d] ($\Delta_1 = 1.384$, 1.536, 2.173, 3.319 × 2, 4.234, 5.331, and 7.041 ms) for the QDEPT-PRJ version (to span a $J_{H,C}$ range of 360−70 Hz); the ^{1}H-selection pulse can be set to an appropriate value (e.g., 45°) as in normal DEPT acquisition or cycled through flip angles 35.3°, 48.0°, 50.6°, 78.5°, 87.5°, and 87.9° as outlined by Jiang et al.[4d] for the QDEPT-PRJ version. In (b) is depicted the DEPT-PRJ.MULT pulse sequence with an optional number of spin echoes ($n = 1, 2,...$ resulting in $2 + 2n$ spin echoes). Similarly, Δ_1 and the ^{1}H-selection pulse can be set (e.g., to $1/(2 \times J_{H,C}$ and 45°, respectively) as in normal DEPT acquisition or cycled methodically for the QDEPT-PRJ.MULT version. In both cases (a and b), Δ_2 can be set independently of Δ_1, typically in the range 1−3 ms and all pulses common to the standard DEPT pulse sequence retained their original phases with the PROJECT pulses within the shaded sections possessing the same phase and phase incrementation as the preceding 180° ^{13}C-pulse (denoted by φ).

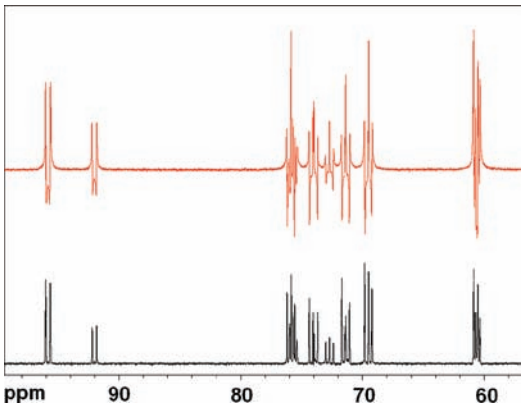


Figure 2. DEPT spectrum of [6-$^{13}C_{1-6}$]-D-glucose (top trace) and the corresponding DEPT-PRJ.MULT spectrum (bottom trace). Conditions: ^{1}H-selection pulse, 45° (both spectra); Δ_1, 3.45 ms (≡ $J_{H,C}$ 145 Hz, both spectra); Δ_2, 2.00 ms; *n*, 3 (8 spin echoes in total); and transients, 32 (both spectra).

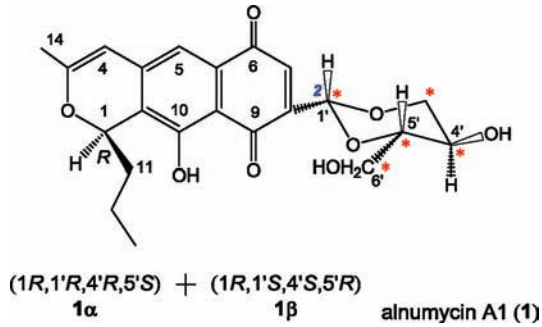


Figure 3. Structures of the inverse epimeric pair constituting (*2*)-(1*R*,1′*RS*,4′*RS*,5′*SR*)-alnumycin A1[7b,c] (**1**) with an indication of the expected label sites (*) arising from the feeding of [5-$^{13}C_{1-5}$]-D-ribose and use of the recently described[9] notation for indicating relative stereochemistry as an extension to the standard Natta projection system;[10] the '*2*' near the C-1′ atom and preceding the name indicates that the number of stereoisomers present in the sample or under consideration is two.

zation for a DEPT 135°-type result could not be accomplished. The signal cost of the experiments varied based on the options taken and the nature of the sample itself, but it was generally minimal and could be contained to within ~10% if the system at hand was well parametrized.

The motivation for the development of DEPT-PRJ arose from a recent biosynthetic sequence study[7a] wherein fully ^{13}C-labeled D-ribose ([5-$^{13}C_{1-5}$]-D-ribose) was fed to a *Streptomyces* bacterial strain resulting in multilabeled metabolites. The inverse epimeric[7b,c] pair constituting (*2*)-(1*R*,1′*RS*,4′*RS*,5′*SR*)-alnumycin[8] A1 (**1**)[7] depicted in Figure 3 was among the metabolites harvested. In order to evaluate incorporation levels and ascertain the intactness of the C_4 unit, various approaches were tried including, in addition to normal carbon observation with the requisite measures in place to ensure reliable quantitation, ^{13}C-edited ^{1}H acquisition, addition of the relaxation agent Cr(acac)$_3$, and DEPT. The latter technique had particular appeal due to the aforementioned reasons, but the perturbation of the multiplets in the multilabeled metabolites arising from ^{13}C homonuclear *J* modulation unfortunately compromised the results and the intactness of the C_4 unit comprising $C_{3'}$–$C_{6'}$ with the labeled carbons originating from C_2–C_5 in [5-$^{13}C_{1-5}$]-D-ribose could not be unequivocally validated directly.

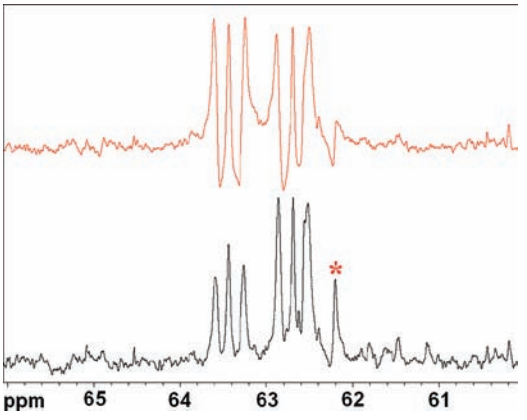


Figure 4. DEPT spectrum of alnumycin A1 (**1**) expanded about the C-6′ carbons {top trace, conditions: ^{1}H-selection pulse, 45°; Δ_1, 3.45 ms (≡ $J_{H,C}$ 145 Hz); and transients, 10k} and the corresponding QDEPT-PRJ spectrum {bottom trace, conditions: ^{1}H-selection pulse, cycled through flip angles 35.3°, 48.0°, 50.6°, 78.5°, 87.5°, and 87.9° as outlined by Jiang et al.;[4d] Δ_1, cycled over 1.384, 1.536, 2.173, 3.319 × 2, 4.234, 5.331, and 7.041 ms values (spanning a $J_{H,C}$ range of 360–70 Hz) as outlined by Jiang et al.;[4d] $\Delta_2 = \Delta_1$; and transients, 18k}. The marked signal (*) is an impurity.

(4) (a) Heikkinen, S.; Toikka, M. M.; Karhunen, P. T.; Kilpeläinen, I. A. *J. Am. Chem. Soc.* **2003**, *125*, 4362. (b) Koskela, H.; Kilpeläinen, I.; Heikkinen, S. *J. Magn. Reson.* **2005**, *174*, 237. (c) Henderson, T. J. *J. Am. Chem. Soc.* **2004**, *126*, 3682. (d) Jiang, B.; Xiao, N.; Liu, H.; Zhou, Z.; Mao, X.-a.; Liu, M. *Anal. Chem.* **2008**, *80*, 8293. (e) Klika, K. D. *Magn. Reson. Chem.* **2010**, *48*, 818. (f) Furrer, J.; Guerra, S.; Deschenaux, R. *Magn. Reson. Chem.* **2011**, *49*, 16.

(5) (a) Bendall, M. R.; Doddrell, D. M.; Pegg, D. T. *J. Am. Chem. Soc.* **1981**, *103*, 4603. (b) Doddrell, D. M.; Pegg, D. T.; Bendall, M. R. *J. Magn. Reson.* **1982**, *48*, 323.

(6) Calibration of the 90° ^{1}H-pulse in the irradiation channel ($^{1}H_{90}$ irr) was accomplished by incrementing all ^{1}H pulses simultaneously (90° + δ, 180° + 2δ) in the DEPT pulse sequence and locating the null point (i.e., the 90° pulse) on a sample of 50% CH_2Cl_2 in $CDCl_3$ under otherwise normal DEPT acquisition conditions (acquisition time, 1 s; postacquisition delay, 3 s; etc.) but optimized on a $J_{H,C}$ value of 177.8 Hz. The 90° ^{13}C-pulse in the observation channel was calibrated prior to calibration of the 90° ^{1}H-pulse using the same sample and locating the null point representing a 360° pulse in the conventional manner.

(7) (a) Oja, T.; Klika, K. D.; Appassamy, L.; Sinkkonen, J.; Mäntsälä, P.; Niemi, J.; Metsä-Ketelä, M. *Proc. Natl. Acad. Sci. U.S.A.*, submitted for publication. (b) Oja, T.; Dreiack, N.; Tähtinen, P.; Mäntsälä, P.; Niemi, J.; Metsä-Ketelä, M.; Klika, K. D. *Chem. Commun.*, submitted for publication. (c) Klika, K. D.; Oja, T.; Dreiack, N.; Mäntsälä, P.; Niemi, J.; Tähtinen, P.; Metsä-Ketelä, M. Unpublished results.

(8) The nomenclature in use follows a recent description[9] whereby the '(*2*)' preceding the name indicates that the number of stereoisomers present in the sample or under consideration is two.

Therefore, application of the PROJECT principle to suppress the *J* modulation in order to obtain normal multiplet intensities was performed and evaluated. An acquisition using the QDEPT-PRJ pulse sequence, with comparison to a normal DEPT acquisition, on alnumycin A1 (**1**) isolated from a culture fed [5-$^{13}C_{1-5}$]-D-ribose is portrayed in Figure 4 whereby it can be seen that considerable spectral improvement was attained. Two stereoisomers are present in the sample, and for each, the C-6′ signal consists of a doublet from incorporation of the fully labeled ribose due to coupling to the C-5′ carbon and a singlet arising from natural abundance. The lines of the doublet are broadened due to long-range couplings to the C-4′, C-3′, and C-1′ carbons. Interestingly, suppression of the C_q magnetization was no longer effective and quaternary carbons were observed elsewhere in the spectra to varying degrees, presumably due to the particular T_1's involved, for various spins.

Thus, it has been successfully demonstrated that incorporation of the PROJECT pulse sequence element for suppression of *J* modulation into the DEPT pulse sequence results in reduction of signal distortion for the multiplets in ^{13}C-multilabeled compounds arising from the $^{13}C-^{13}C$ scalar coupling. This application should be a valuable aid in situations where quantitation is important and cases of ^{13}C-multilabeled compounds arise, e.g. as a result of feeding experiments in biosynthetic studies. By utilizing the QHSQC principle[4] the need for control samples can be circumvented, a concern for cases where signal intensity arising from natural abundance isotopomers can be vanishingly small for sample-limited controls.

Acknowledgment. The Turku University Foundation is thanked for financial support of this study.

Supporting Information Available. Pulse sequence files (in Teutonic format): DEPT pulse sequence for the calibration of $^1H_{90}$ irr; DEPT-PRJ and QDEPT-PRJ pulse sequences (with cycling of the interpulse delays and ^1H-selection pulse for rendering signal response invariant to *J* over a prescribed range), both with variants for additional spin echoes (DEPT-PRJ.MULT and QDEPT-PRJ.MULT); and example text files for variable interpulse delays and ^1H-selection pulses. This material is available free of charge via the Internet at http://pubs.acs.org.

(9) Klika, K. D. *Int. J. Org. Chem.* **2011**, *1*, 215.
(10) Giulio Natta, 1903–1979.