Heteronuclear Long Range Chemical Shift Correlation Using the INEPT-DANTE Experiment

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Abstract: *Selective INEPT experiment INEPT-DANTE was created by substitution of all selective soft rectangular pulses of the INAPT sequence by equivalent DANTE pulse trains. The INEPT-DANTE experiment was shown to be more selective than INAPT by applying both sequences to the simple compound 2-ethoxybenzaldehyde.*

Introduction

Information on heteronuclear long-range chemical shift correlation (HETCOR) NMR spectroscopy is very important for the structure determination and correct assignment of resonances of all types of molecules. One of the techniques that have been used to obtain such information is the selective version of INEPT[1] ('Insensitive Nuclei Enhanced by Polarization Transfer'). The INAPT sequence[2], the selective version of INEPT, has been used in the structure determination of natural products[3] and in the study of nucleic acids and nucleosides.[4] The advantage of this experiment resides in the fact that it is a one-dimensional experiment which functions through selective polarization transfer via heteronuclear long range scalar coupling. Several modern NMR techniques, such as HETCOR,[5] COLOC,[6] and HMBC[7] also afford heteronuclear long range correlations, but they often fail when the coupling path includes one heteroatom. However, INAPT is especially useful to establish three-bond correlations in such situations.[4]

The INEPT pulse sequence uses non-selective polarization transfer from sensible and abundant nuclei (A) to less sensible diluted nuclei (X) by J_{XA} modulation. In the refocused version,[8] the anti-phase magnetization components precess into phase before acquisition, allowing broad band decoupling of the sensitive nuclei during acquisition, which leads to an enhanced all-singlet spectra. Traditionally, the INAPT experiment is carried out by replacing all nonselective proton pulses in the refocused INEPT experiment by rectangular soft pulses ($\gamma B_2/2\pi \approx 25$ Hz). These selective pulses are obtained by increasing the pulse length and attenuating the rf power (i.e. the pulse amplitude). The principle behind the INAPT experiment is that if the rf field strength of the soft pulse at the frequency of the A nucleus, expressed in hertz, is much smaller than the direct J_{XA} coupling constant ($\gamma B_2/2\pi << {}^{1}J_{XA}$), the X satellites will not be affected by soft pulses. In this way, the resonance of the X nucleus, which is directly coupled to the selected A nucleus, will not appear in the INAPT spectrum. It is thus possible to obtain carbon-hydrogen long-range correlations even for quaternary carbons. Satisfactorily selective results are obtained with this technique only when the attenuation of rf power is strong enough ($\gamma B_2/2\pi \approx 25$ Hz) to affect a very

narrow frequency range, avoiding additional ^{13}C satellite excitation due to heteronuclear one-bond coupling. The use of narrow excitation bands obtained with soft rectangular pulses may not guarantee the determination of correct correlations, especially because the selectively excited hydrogen may be too close to one or several other hydrogens. The ideal solution for this selectivity problem would be the use of shaped pulses,[9] but this option is not available in many spectrometers. Therefore, other solutions must be adopted.

We propose the utilization of the INEPT sequence with selective rectangular pulses obtained by DANTE[10,11] ('Delays Alternating with Nutation for Tailored Excitation') pulses in order to obtain better selectivity with spectrometers that do not have a wave form generator. DANTE is a pulse sequence obtained by application of n very short identical hard pulses with a nutation angle $\alpha << \pi/2$, separated by a very short time delay tau (τ). The DANTE pulse train can be obtained by application of the sequence $(\alpha - \tau)_n$, where n is the total number of hard pulses in the DANTE sequence, and $n\alpha$ is equal to θ, the total nutation angle for the DANTE pulse train. In practice, the duration Δt of each pulse of the DANTE sequence is much shorter than the time interval between pulses ($\Delta t << \tau$) so that the total duration of the DANTE pulse train can be considered equal to $n\tau$. In this way, the selectivity of the DANTE pulse train is controlled by the total number of pulses (n) and by the time delay (τ), and not by the degree of rf attenuation. Due to limitations on the spectrometer hardware, there is a limit to rf attenuation. DANTE provides a different means to obtain selectivity, without needing much power attenuation, which is very convenient to implement in the INAPT

experiment. In this work, we have created an INEPT sequence with DANTE, INEPT-DANTE, and compared its selectivity with the conventional INAPT experiment, using *o*-ethoxybenzaldehyde (**1**) as the sample.
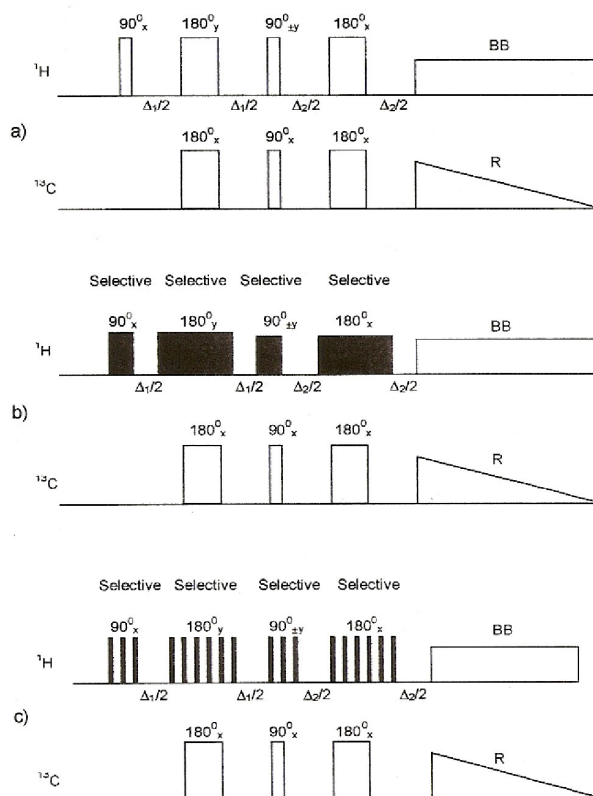
Experimental

One-dimensional ^{13}C and ^{1}H NMR spectra were acquired on a Varian UNITY-300 NMR spectrometer operating at 75 and 300MHz respectively. The ^{13}C 90^0 pulse used was 11.4μs. ^{13}C pulses, all of which hard, were set at the middle of soft ^{1}H pulses. Polarization transfer experiments from ^{1}H to ^{13}C using small three-bond couplings were carried out using the INAPT pulse sequence as described by Bax[2] and our version using DANTE pulses for selective excitation. The calibration of 1H pulses was made using the procedure and pulse sequence described by Bax.[12] These calibrations were obtained using a sample of dichloroacetic acid in CDCl$_3$ for the INAPT pulse sequence, and chloroform in deuterated acetone for the INEPT-DANTE, obtaining 90^0 pulses of 7285μs and 67μs respectively. For selective excitation, the hydrogen pulse transmitter frequency was centered on the resonance of interest. The DANTE pulse was obtained by application of 90 hard pulses (n=90) separated by a delay (τ) of 741μs. The time intervals Δ_1 and Δ_2, which correspond to $1/2J_{CH}$, were optimized to allow polarization transfer via $^{3}J_{CH}$ coupling ($^{3}J_C$=10Hz). The experiments were performed on a sample of 50% *o*-ethoxybenzaldehyde in DMSO-d_6 in a 5mm sample tube at 19 ^{0}C and were acquired with 128 scans with a relaxation delay of 2s.

Results and Discussion

Compound 1 was chosen for this work, among many other possible compounds, because its ^1H and ^{13}C NMR signal assignment is well known. Also, its aromatic hydrogens possess very close δ values with no superposition of hydrogen resonance central lines with ^{13}C satellites, allowing us to test the selectivity of the pulses. The INAPT pulse sequence was obtained by the amplitude attenuation of all decoupler pulses present in the refocused INEPT pulse sequence. In order to obtain the INEPT-DANTE pulse sequence the decoupler pulses of the refocused INEPT pulse sequence were substituted by DANTE pulse trains with the desired total nutation angle (Fig. 1). Since DANTE is not a standard

pulse sequence in most spectrometers; it was necessary to design and implement an adequate program, which included the desired alternated rectangular hard pulses and time delays.[13] The design of this program was carried out in such a way as to allow the easy control of selectivity by means of the interactive manipulation of parameters n and τ. For comparison of the performance of the two pulse sequences the relation between pulse attenuation and the obtained selectivity was established. This relation is important because rectangular pulses provide excitation bandwidths narrow enough to affect only a single resonance when pulse attenuation is strong, i.e. if pulse width is very large.


Figure 1. Pulse Sequence scheme: a) refocused INEPT, b) INAPT and c) INEPT-DANTE

The rf field strength used in the soft pulses of the INAPT experiment ($\gamma B_2/2\pi \cong 34Hz$) was much less than that used in the INEPT-DANTE experiment ($\gamma B_2/2\pi \cong 3731Hz$). In order to refocus ^{13}C magnetization to allow hydrogen decoupling during acquisition, time intervals, Δ_1 and Δ_2 ($1/2J_{CH}$) were set in such a way as to make polarization transfer possible via $^3J_{CH}$ coupling ($^3J_{CH} = 10Hz$).

Closeness between NMR resonances of the aromatic hydrogens of the sample used (Fig. 2).
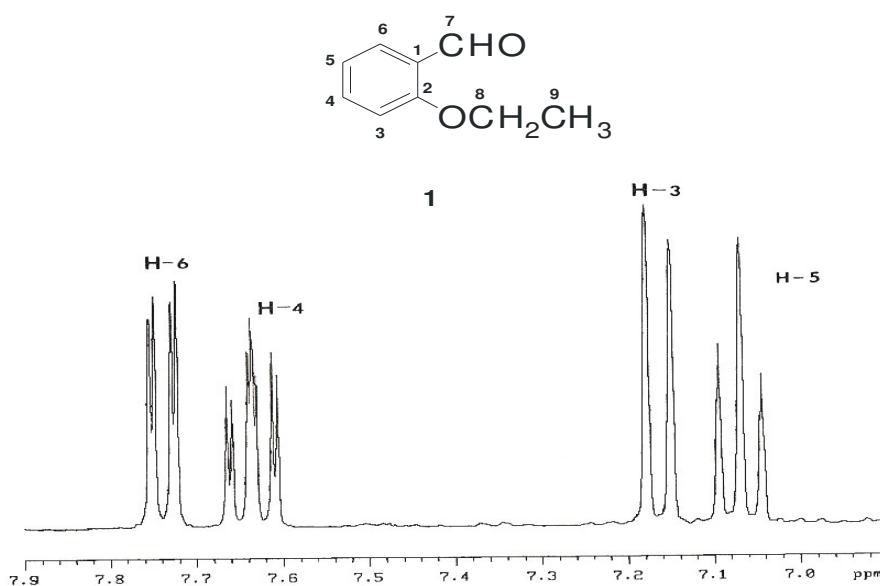
Figure 2. Expansion of the aromatic region of the ^1H NMR spectrum of **1** showing the resonances of H-3, H-4, H-5 and H-6.

The INAPT spectrum with the selective excitation of H-4 is shown in Fig. 3b. As delays $\Delta 1$ and $\Delta 2$ were optimized for polarization transfer *via* $^3J_{CH}$. This spectrum would be expected to show only resonances for C-2 and C-6, but we also see peaks for all the other sp^2 carbons of 1, including C-7 (inverted). In this experiment, polarization transfer to C-3 and C-5 could be possible if $^2J_{CH} \approx {}^3J_{CH}$, but the resonance for C-7 would mean that a highly improbable polarization transfer through five bonds coupling would have occurred. The plausible explanation for this correlation is that the INAPT soft pulse also excited H-6 or one of its ^{13}C satellites. Similarly, the appearance of signal of C-4 would mean that there was some excitation either of H-4 satellite bands due to $^1J_{CH}$ coupling with C-4 or of H-6. Indeed, these results are explained by the lower selectivity of the soft pulses of the INAPT experiment. In contrast, Fig. 3c shows the spectrum obtained by selective excitation of H-4 with the INEPT-DANTE experiment, in which only the expected C-2 and C-6 resonances appear.

The INAPT experiment with selective excitation of H-6 (Fig. 3d) gives a better result when compared to the same experiment with H-4 (Fig. 3b). As can be seen, the resonance for H-6 is further away from those of H-3 and H-5 than H-4. But even in this case, the INAPT spectrum with excitation of H-6 shows, along with the expected resonances, the signal of C-3. Clearly, the lower selectivity of the soft rectangular pulse of INAPT is exciting, besides the resonance of H-6, one of the ^{13}C satellites of H-3 or the resonance of H-4. In contrast, the INEPT-DANTE experiment with H-6 excitation is very clean and shows only the resonances of carbon atoms that are three bonds away from H-6, which are C-7, C-2 and C-4 (Fig. 3e). The same type of results is obtained when both experiments are carried out with the selective excitation of H-3 and H-5.

From these results we have found that the INEPT-DANTE experiment is more selective than INAPT, giving clear cut results even in cases where hydrogen resonances are only 0.1ppm apart.
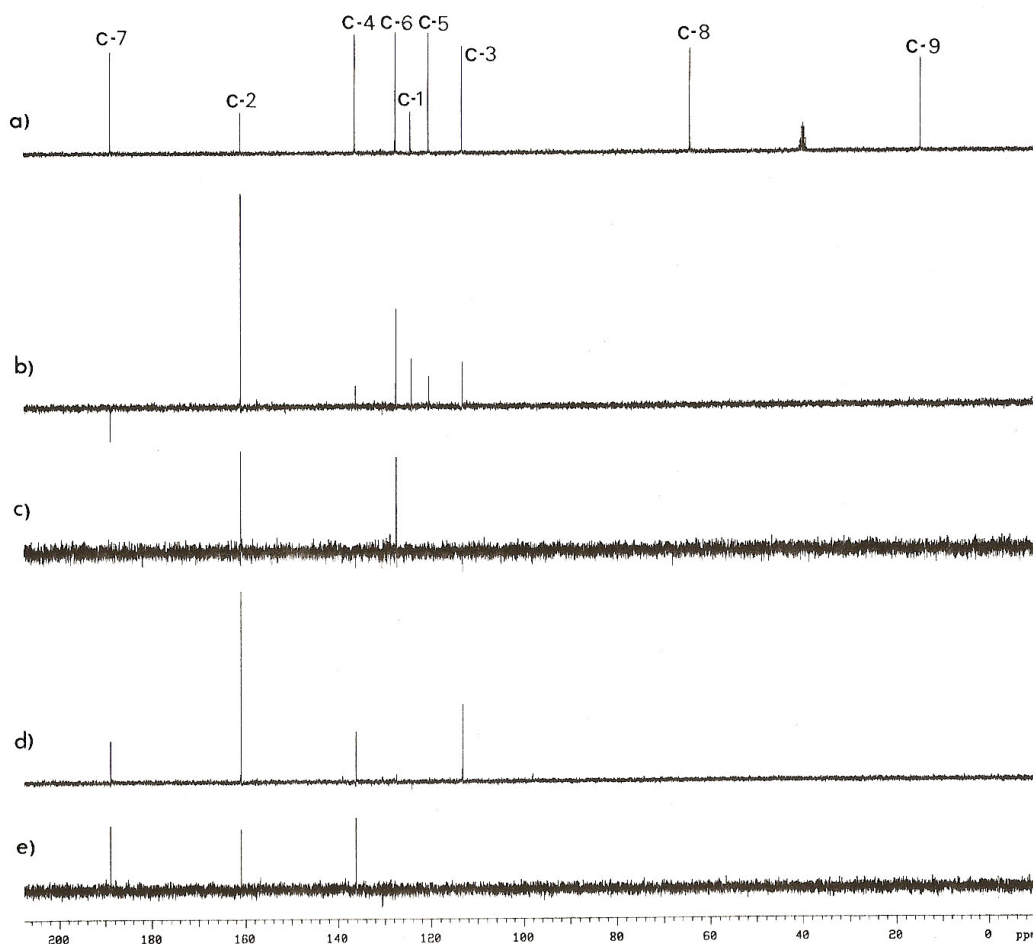
Figure 3. Spectrum of **1** obtained with different experiments. a) ^{13}C NMR; b) INAPT with selective excitation of H-4; c) INEPT-DANTE with selective excitation of H-4; d) INAPT with selective excitation of H-6 and e) INEPT-DANTE with selective excitation of H-6.

Conclusions

For INEPT-DANTE, the rf pulse field strength $\gamma B_2/2\pi \cong 3731$ Hz was sufficient to obtain a spectral excitation band of 30 Hz, given by $2/n\tau$ ($n\tau \approx 67$ms). Using rectangular soft pulses, this kind of selectivity could only be accomplished using a pulse length of 67 ms with a very strong rf pulse field strength attenuation ($\gamma B_2/2\pi \cong 3.75$ Hz), which in most spectrometers would mean the installation of additional rf attenuators. The selective decoupler rectangular pulse used in the INAPT pulse sequence with $\gamma B_2/2\pi \cong 34$Hz furnished a spectral excitation band, given by $2/t_p$ ($t_p = 7.3$ms), equal to 274Hz. Regarding excitation selectivity, the INEPT-DANTE sequence is clearly superior to INAPT, specially when the molecule in study has very close hydrogen resonances (≈ 0.1 ppm). The fact that the pulse duration of DANTE is not controlled by the rf pulse field strength, but by $n\tau$ value, makes it possible to obtain selective excitation bands. As INEPT is a one-dimensional pulse sequence, we believe that the INEPT-DANTE experiment is an important tool for rapidly solving specific signal assignments or structural problems by NMR.

Acknowledgment

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13. The INEPT-DANTE program for UNITY-300 is available by request to figueroa@ime.eb.br.