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The Geometrical Imprint Domain: A 1D and 2D Spectra Editing Tool in Refocused–Decoupled INEPT Experiments for Structural Analysis of Silylated and Acetylated Glycosides

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The influence of homonuclear coupling contributions in ^{13}C and ^{29}Si NMR refocused–decoupled 1D/2D INEPT experiments was graphically visualized by monitoring the computed variations of the theoretical signal intensities as a function of the refocused delay time Δ. The appearance and exploitation of a hitherto unknown characteristic 'geometrical imprint domain' closely linked to the spatial environment around the heteroatom under investigation allow a straightforward editing of the spectra. © 1997 John Wiley & Sons, Ltd.

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INTRODUCTION

Numerous investigations have focused on empirical and experimental assignment methods for ^{29}Si NMR structural determinations in carbohydrate chemistry.[1] Difficult analytical problems encountered due to the inherent low sensitivity of the ^{29}Si nucleus have been circumvented by the use of routine polarization transfer techniques.[2] Soon after the introduction of the INEPT sequence,[3] Doddrell *et al.*[4] and Burum and Ernst[5] derived the analytical expressions for the theoretical enhancement of decoupled INEPT spectra where the only parameters to be manipulated are the delay times τ and Δ. From a practical point of view, the fundamental parameter proved to be the refocusing delay time Δ, which may be set to obtain optimum enhancement or selectively to invert or suppress specific resonances.[6] Nevertheless, so far no general editing method for ^{29}Si and quaternary ^{13}C NMR spectra have been described, taking into account the influence of the contributions of both hetero- and homonuclear couplings.

To quantify the involvement of homonuclear couplings in the magnitude of the enhancement factor, Schenker and von Philipsborn[7] introduced a correction term, $\cos[(\pi\tau J(\mathrm{S}^p, \mathrm{S}^q)]$, in the general equation describing the obtainable enhancement factor $E_{\mathrm{INEPT\ dec.}}$ of the I-multiplet components of an arbitrary spin-$\frac{1}{2}$ system $\mathrm{IS}^1\mathrm{S}^2\mathrm{S}^3$ as a function of both the INEPT time parameters and the contribution of additional sets of equivalent or non-equivalent hydrogens:

$$\begin{aligned} E_{\mathrm{INEPT\ dec.}}(\tau, \Delta) = \gamma_{\mathrm{S}}/\gamma_{\mathrm{I}} \sum^{p} & \sin[\pi\tau J(\mathrm{I}, \mathrm{S}^p)] \\ & \times \sin[\pi\Delta J(\mathrm{I}, \mathrm{S}^p)]\Pi^{q(q\neq p)} \\ & \times \cos[\pi\tau J(\mathrm{S}^p, \mathrm{S}^q)] \\ & \times \cos[\pi\Delta J(\mathrm{I}, \mathrm{S}^q)] \end{aligned} \qquad (1)$$

where p and q represent the 1, 2, 3, ... indices of the S^1, S^2, S^3, ... nuclei in correlation systems.

In order to take advantage of all the potential of the refocused INEPT expression, we developed a computed graphical representation $E_{\mathrm{INEPTdec}} = f(\tau, \Delta)$ of Eqn (1), which takes into account the contributions of the different heteronuclear $^nJ_{\mathrm{X-H}}$ couplings in the environment of a given spin-$\frac{1}{2}$ nucleus.[8] Owing to the critical choice of the polarization delay τ in INEPT experiments, the variations of the enhancement E_{d} have to be recorded for the two characteristic and optimized τ values ascribable to each heteronuclear contribution in the environment of the nuclei. We recently determined these parameters by non-refocused INEPT experiments on the application of the 2D spin-flip J-resolved technique

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to the structural analysis of trimethylsilylated glycosides ($\tau = 0.0357$ s for $^2J_{\text{Si–H}} = 6.6$–6.7 Hz and $\tau = 0.06$ s for $^3J_{\text{Si–H}}$ varying from 2.1 to 4.3 Hz).[9]

In the absence of $^3J_{\text{Si–H}}$ and $^3J_{\text{H–H}}$ couplings, graphs of decoupled enhancements for an arbitrary silicon system with $^2J_{\text{Si–H}} = 6.6$ Hz, $9 < n < 12$ and $\tau = 0.0357$ s, closely parallel conventional plots resulting from the Doddrell and Ernst expressions and show the same dependence of their relationships to the number of protons involved. Opposite amplitude modulations were observed for silicon nuclei coupled to an odd/even number of protons at large Δ values ($0.125 < \Delta < 0.187$) [Fig. 1, $E_{\text{INEPTdec}} = f(\Delta)$: n even (10 protons, full line; 12 protons, small dashed line); n odd (11 protons, dotted line)].

In the presence of additional heteronuclear $^3J_{\text{Si–H}}$ long-range couplings and homonuclear $^3J_{\text{H–H}}$ vicinal couplings, the corresponding plot (long dashed line) reveals significant modifications due to a drastic shift of the previous time interval to larger Δ values ($0.250 < \Delta < 0.375$) and the appearance of additional low-amplitude modulations in the first $0.125 < \Delta < 0.187$ delay time interval ($E_{\text{INEPTdec[Si-2]}} = f(\Delta)$ in the model compound methyl 2,3,4,6-tetrakis-*O*-trimethylsilyl-*β*-D-galactopyranoside: long dashed line in Fig. 1).

This sensitivity to additional $^3J_{\text{Si–H}}/^3J_{\text{H–H}}$ coupling effects should give a base for analytical applications of the refocused–decoupled 1D INEPT technique in the elucidation of the structure of silylated compounds and spectral editing capability. Hence the question which arises is whether the spatial arrangement around silicon nuclei could be directly related to the presence of this extra modulation domain (long dashed line, $0.125 < \Delta < 0.187$), giving rise to striking and exploitable properties of the graphs, such as amplitude modulations.

RESULTS AND DISCUSSION

By analogy with previous studies in carbohydrate chemistry,[9] investigations were performed on the model compounds methyl 2,3,4,6-tetrakis-*O*-trimethylsilyl-*α*-D-manno- (**1**), — *α*-D-gluco- (**2**) and — *β*-D-galactopyranoside (**3**). Superposition of the whole $E_{\text{INEPTdec}} = f(\Delta)$ curves displays the characteristic Δ intervals allowing direct assignment of silicon nuclei in corresponding decoupled spectra for a determined τ value. It became apparent that the general shape of each refocused INEPT representation in the $0.125 < \Delta < 0.187$ domain depends on the $^3J_{\text{H–H}}$ values of vicinal protons close to each silicon atom [$J_{\text{H–H(axial/axial)}}$, $J_{\text{H–H(axial/equatorial)}}$ and $J_{\text{H–H(equatorial/equatorial)}}$].

In Fig. 2, the superposition of time intervals is enlarged, leading to discrimination between silicon systems where n is odd or even [Fig. 2(a) $\tau = 0.0357$ and Fig. 2(b) $\tau = 0.06$ s]. The refocusing time was varied between 0.225 and 0.375 s. In order to recognize directly the influence of $J_{\text{H–H(axial/axial)}}$, $J_{\text{H–H(axial/equatorial)}}$ and $J_{\text{H–H(equatorial/equatorial)}}$ proton–proton couplings, we arbitrarily recorded graphs using full lines for two high $J_{\text{H–H}}$ values [$J_{\text{H–H(axial/axial)}}/J_{\text{H–H(axial/axial)}}$ varying in the range 7–9.3 Hz], dotted lines for two small $J_{\text{H–H}}$ values [$J_{\text{H–H(axial/equatorial)}}/J_{\text{H–H(axial/equatorial)}}$ or $J_{\text{H–H(axial/equatorial)}}/J_{\text{H–H(equatorial/equatorial)}}$ varying from 1 to 2.5 Hz] and dashed lines for high and small $J_{\text{H–H}}$ values [$J_{\text{H–H(axial/axial)}}/J_{\text{H–H(axial/equatorial)}}$ varying from 1.8 to 9 Hz]. Dot-dashed lines are ascribable to the Si-6 silicon systems (CH_2OSiMe_3). For $\tau = 0.0357$ s and $0.268 < \Delta < 0.294$, silicons coupled with 11 protons (CH_2OSiMe_3) are observed with negative phase, while silicons coupled with 10 protons ($CHOSiMe_3$) are

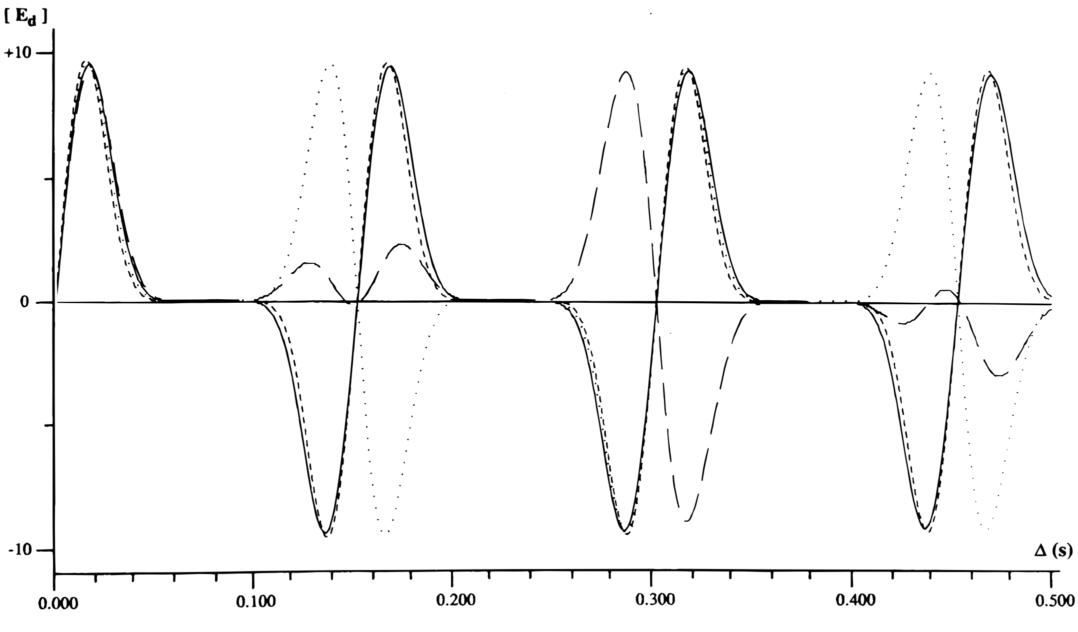


Figure 1. Graphs of decoupled enhancements, $E_{\text{INEPTdec.}} = f(\Delta)$, showing the dependence of that relationship on the number of protons implied for $\tau = 0.0357$ s. Where $^2J_{\text{Si–H}} = 6.6$ Hz: full line for $n = 10$, small dashed line for $n = 12$, dotted line for $n = 11$. Where $^2J_{\text{Si–H}} = 6.6$ Hz, $^3J_{\text{Si–H}} = 3.4$ Hz, $^3J_{\text{H–H}} = 9.2$ Hz, $^3J'_{\text{H–H}} = 7.4$ Hz: dashed line [in methyl 2,3,4,6-tetrakis-*O*-trimethylsilyl-*β*-D-galactopyranoside (**3**)].

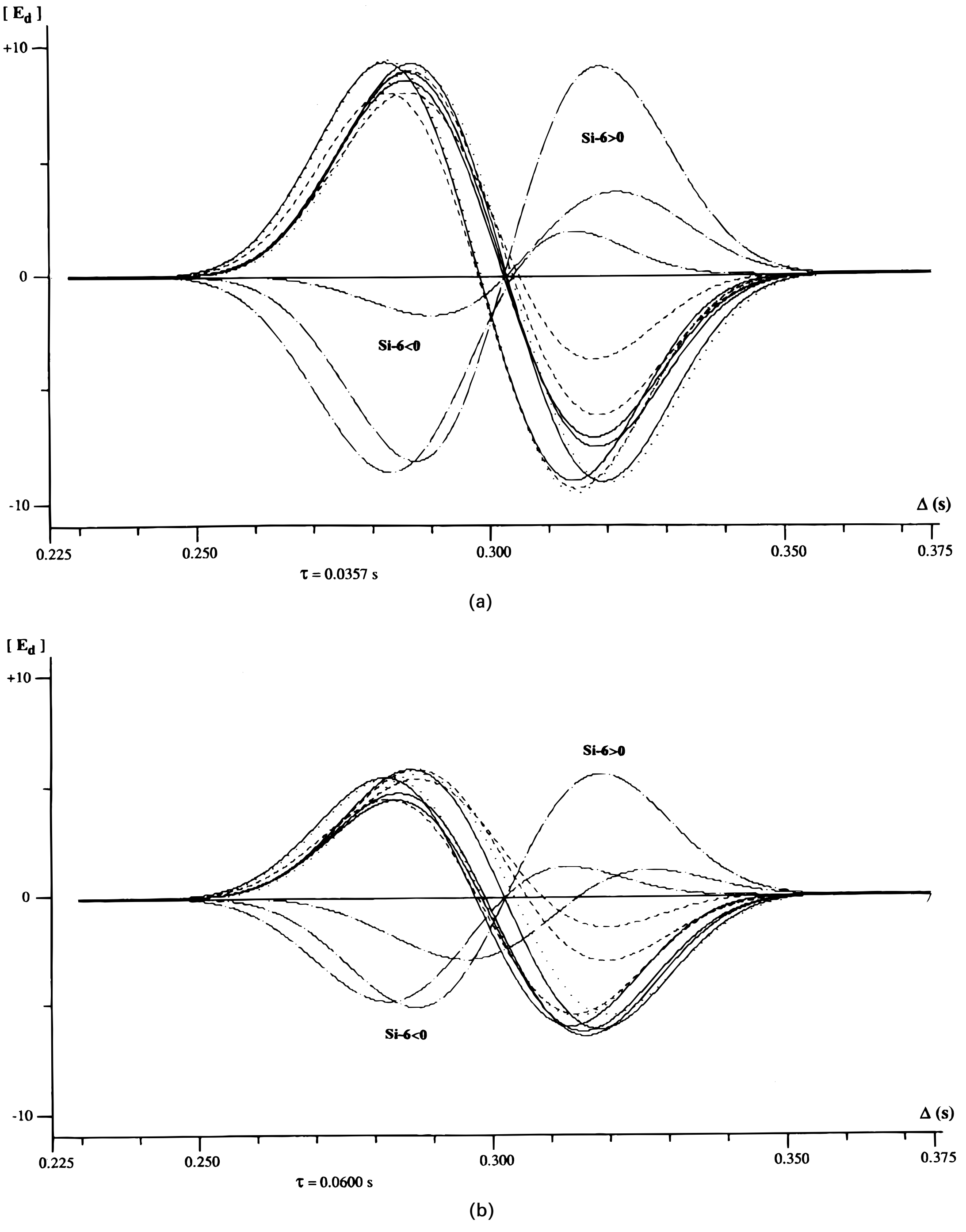


Figure 2. Zoom of decoupled enhancements ($E_{\mathrm{INEPTdec.}}$) versus Δ values in the 0.225–0.375 s window for (a) $\tau = 0.0357$ and (b) 0.06 s. J_{aa}/J_{aa} [$J_{aa} = {}^3J_{\mathrm{H(axial)-H(axial)}}$ vicinal coupling constant values], full lines; J_{ae}/J_{ae} or J_{ae}/J_{ee}, dotted lines; J_{aa}/J_{ae}, dashed lines; Si-6 system, dot-dashed lines for compounds **1**–**3**.

observed with positive phase. Opposite signs result from larger Δ values ($0.306 < \Delta < 0.334$). Analogous information is provided from $\tau = 0.06$ s ($E_{\mathrm{Si\text{-}6}} < 0$ for $0.268 < \Delta < 0.294$ and $E_{\mathrm{Si\text{-}6}} > 0$ for $0.315 < \Delta < 0.328$). In practice, as $E_{\mathrm{INEPTdec.}}$-computed representations do not take into consideration relaxation effects due to an $e^{-\Delta/T_2}$ exponential factor, the most reliable Δ domain in determining amplitude inversions is the first one ($0.268 < \Delta < 0.294$).

The intermediate delay time range lying between 0.075 and 0.240 s gives rise to amplitude modulations depending on the presence of different heteronuclear couplings as well as homonuclear couplings associated with the ${}^3J_{\mathrm{Si-H}}$ system. Graphs of theoretical enhancements in the 0.075–0.240 s Δ window are shown in Fig. 3 [(a) $\tau = 0.0357$ and (b) $\tau = 0.06$ s].

The following observations could be made. In the local environment of the silicon atom under investigation where ${}^3J_{\mathrm{H-H}}$ vicinal couplings are axial/equatorial and axial/equatorial or axial/equatorial and equatorial/equatorial (${}^3J_{\mathrm{H-H}}$ varying from 1 to 2.5 Hz), the amplitude modulation of the signal is negative in the $0.152 < \Delta < 0.174$ range for $\tau = 0.0357$ s. In the same way, the Si-6 modulation (CH_2OSiMe_3) could appear

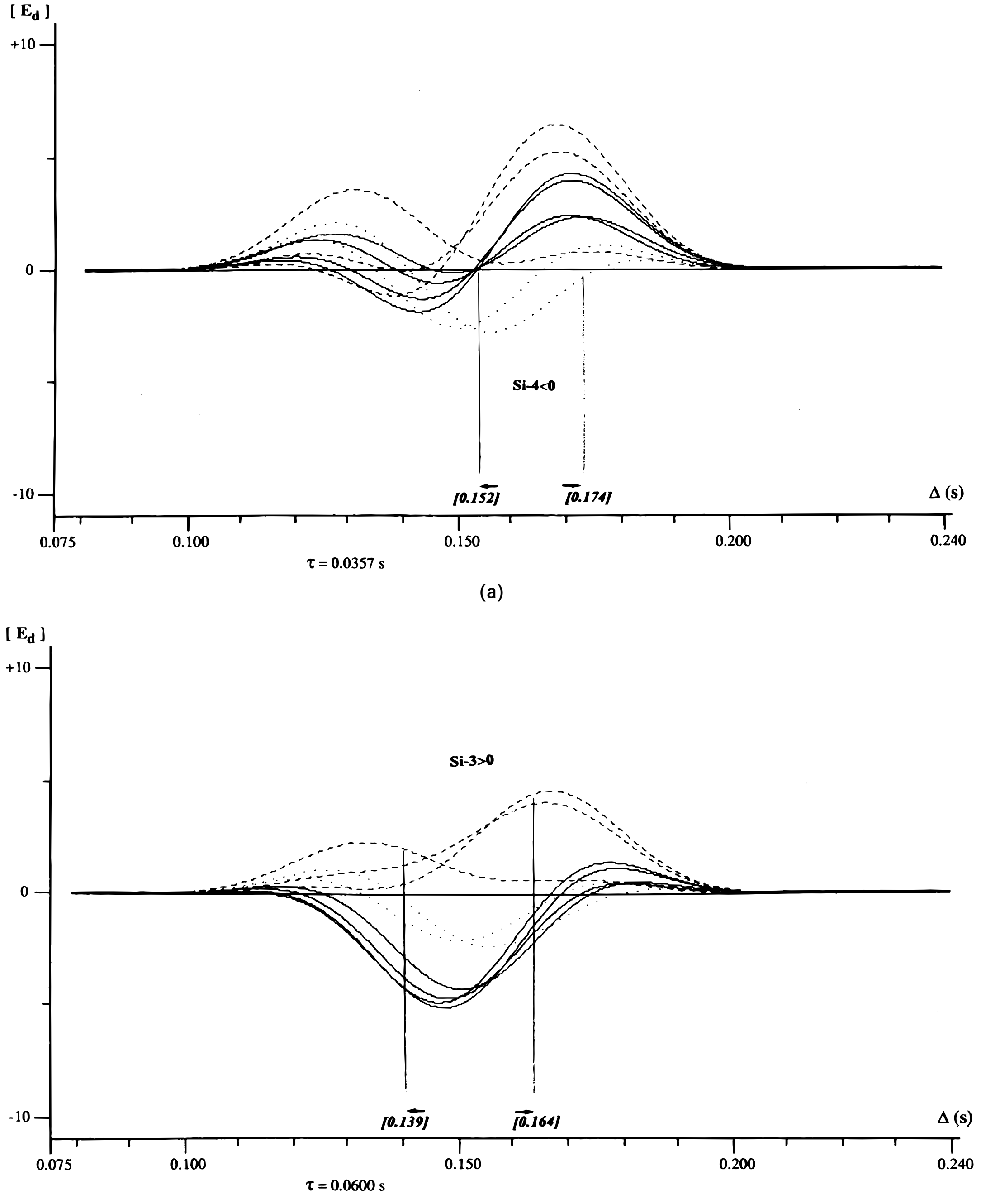
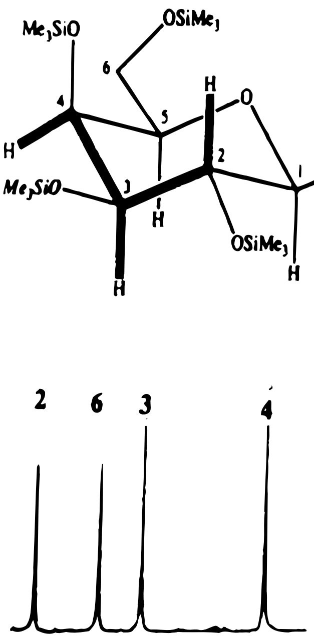


Figure 3. Zoom of decoupled enhancements ($E_{\text{INEPTdec.}}$) versus Δ values in the 0.075–0.240 s window for τ = (a) 0.0357 and (b) 0.06 s using the same agreements as in Fig. 2 for compounds **1**–**3**.

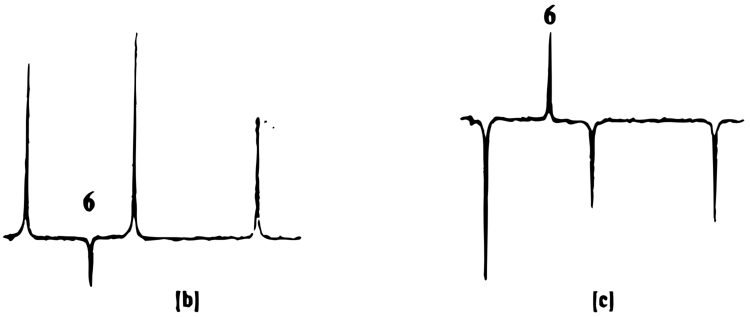
negative, which is not a problem as assignment could be directly deduced from the $0.268 < \Delta < 0.294$ time interval. Where ${}^3J_{\text{H–H}}$ vicinal couplings are axial/axial and axial/equatorial (${}^3J_{\text{H–H}}$ varying from 7.4 to 9.2 Hz and from 1 to 2.5 Hz), the amplitude modulations are positive in the $0.139 < \Delta < 0.164$ range for $\tau = 0.06$ s. Thus, observed characteristic Δ values in the 0.075–0.240 s range proved to be closely linked to the vicinal proton–proton linkage geometry. We call this Δ delay time window the 'geometrical imprint domain.'

Representative refocused INEPT spectra of methyl 2,3,4,6-tetrakis-*O*-trimethylsilyl-*β*-D-galactopyranoside (**3**) are depicted in Fig. 4, in which the refocusing time Δ was varied in order to invert selectively silicon resonances.

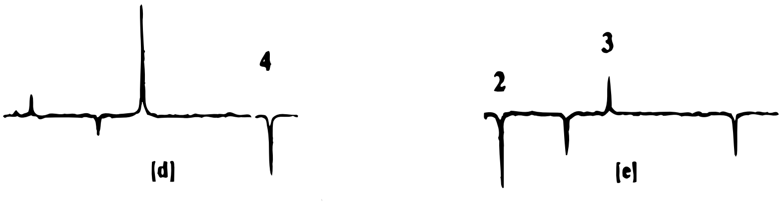
First, we determine classically the number of silicon atoms involved for $\tau = 0.0357$ s and $\Delta = 0.02$ s (a). Subsequently, the Si-6 signal is localized using $\tau = 0.0357$ s and $\Delta = 0.2896$ s (b). The signal returns to a positive value for $\tau = 0.0357$ s and $\Delta = 0.32$ s (c). Finally, the Si-4 signal is assigned for $\tau = 0.0357$ s and $\Delta = 0.16$ s (d), where only Si-6 and Si-4 are negative. The positive intensity of Si-3 for $\tau = 0.06$ s and $\Delta = 0.145$ s allows one to distinguish between Si-3 and Si-2 nuclei (e). All the compounds **1**–**3** can be gradually assigned in this way. Obviously, the method is of limited applicability



[a] Experimental spectrum obtained for $\tau = 0.0357s$ and $\Delta = 0.020s$ (optimal enhancement)



Experimental spectra obtained for [b]: $\tau = 0.0357s$, $\Delta = 0.2896s$ and [c]: $\tau = 0.0357s$, $\Delta = 0.3200s$, according to the odd/even number of heteronuclear ^{29}Si-^{1}H couplings.



Experimental spectra obtained for [d]: $\tau = 0.0357s$, $\Delta = 0.1600s$ and [e]: $\tau = 0.06s$, $\Delta = 0.1450s$, according to the contribution of homo and heteronuclear couplings [geometrical imprint domain].

Figure 4. Representative refocused INEPT spectra editing method on methyl 2,3,4,6-tetra-*O*-trimethylsilyl-*β*-D-galactopyranoside (**3**) for predetermined Δ values.

when vicinal protons have identical coupling constants. These results concern definite Δ domains and are only valuable for the described homogeneous series, where $^{3}J_{\mathrm{Si-H}}$ heteronuclear couplings remain constant throughout the series. Application of the method to other types of compounds or nuclei requires a redefinition of the τ and Δ domains from computed $E_{\mathrm{INEPTdec.}} = f(\tau, \Delta)$ representations.

The results available so far allow us to extend the same approach to the model 1,3,4,6-tetra-*O*-acetyl-*β*-D-mannopyranose (**4**) (Fig. 5).[10] Selective acetylation of the hydroxyl functions is a very commonly used introduction of protective groups in carbohydrate chemistry.[11] Several studies concern the acetylation regioselectivity control although major difficulties can be found in the assignment of the acetyl NMR signals.

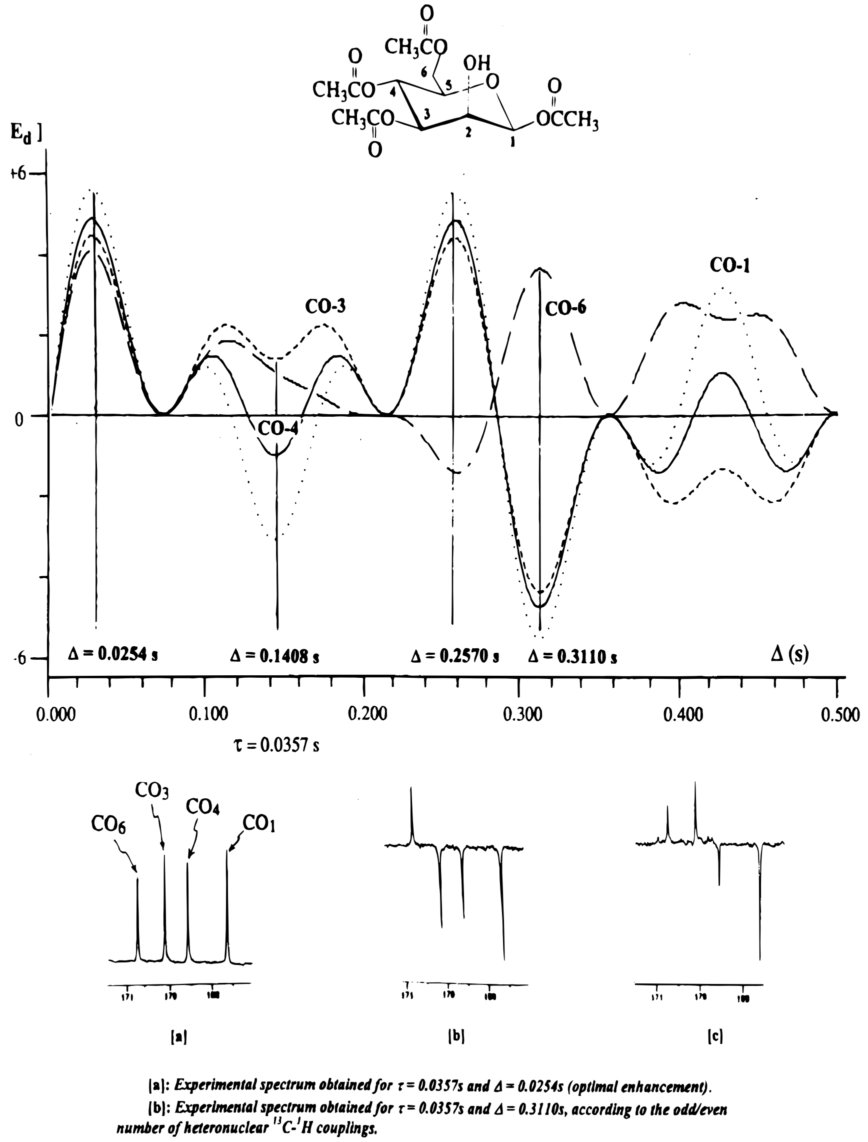
As expected from theoretical lines (Fig. 5), spectral editing and identification of acetyl NMR signals could be directly inferred from significant amplitude modulations of sp^2 carbon atom resonances, according to characteristic Δ values.[12] Resonance assignment between CO-1 and CO-4 systems was based on their theoretical relative signal intensities in the 'geometrical imprint domain' for $\Delta = 0.1408$ s (CO-6, dashed line; CO-3, small dashed line; CO-4, full line; CO-1, dotted line).

Nevertheless, keeping in mind that theoretical $E_{\mathrm{INEPT.dec}} = f(\tau, \Delta)$ graphical representation of a specific CO resonance reports the sum of all ${}^2J_{\mathrm{C(O)-CH}}$ contributions between the sp^2 carbon atom and the methyl hydrogens on the one hand and ${}^3J_{\mathrm{C(O)-O-CH}}$ contributions [modulated by $\sum$(H,H) couplings] between the sp^2 carbon atom and the vicinal cyclic protons on the other, an objection can be raised that resonance assignments based on relative signals intensities in the 'geometrical imprint domain' give rise to spurious responses arising from the presence of the ${}^2J_{\mathrm{C(O)-CH}}$ contributions. Against this objection, we separately recorded the ${}^2J_{\mathrm{C(O)-CH}}$ and ${}^3J_{\mathrm{C(O)-O-CH}}$ contributions from the global $E_{\mathrm{INEPT.dec}}$ representation.

The intensities of the modulations, characterized by the constant

$$k_i = \sin[\pi\tau J(\mathrm{C,H})]\sin[\pi\Delta J(\mathrm{C,H})]\Pi^{q(q\neq p)} \times \cos[\pi\tau J(\mathrm{H,H})]\cos[\pi\Delta J(\mathrm{H,H})]$$

clearly establish that for $\Delta = 0.1408$ s, the ${}^2J_{\mathrm{C(O)-CH}}$ contribution is equal to zero ($k_i = 0$). Hence no line intensity distorsions have to be taken into account and the



|a|: Experimental spectrum obtained for τ = 0.0357s and Δ = 0.0254s (optimal enhancement).

|b|: Experimental spectrum obtained for τ = 0.0357s and Δ = 0.3110s, according to the odd/even number of heteronuclear ${}^{13}C$-${}^{1}H$ couplings.

|c|: Experimental spectrum obtained for τ = 0.0357s and Δ = 0.1408s, according to the contribution of homo and heteronuclear couplings [geometrical imprint domain].

Figure 5. Graphs of decoupled enhancements, $E_{\mathrm{INEPTdec.}} = f(\Delta)$, for $\tau = 0.0357$ s in 1,3,4,6-tetra-*O*-acetyl-β-D-mannopyranose (**4**), where ${}^3J_{\mathrm{CO-O-C}H} = 3.5$ Hz and ${}^2J_{\mathrm{CO-C}H} = 7$ Hz (${}^3J_{\mathrm{H1-H2}} = 1.1$ Hz; ${}^3J_{\mathrm{H2-H3}} = 3$ Hz; ${}^3J_{\mathrm{H3-H4}} = 9.8$ Hz; ${}^3J_{\mathrm{H4-H5}} = 9.8$ Hz; ${}^3J_{\mathrm{H5-H6a}} = 4.9$ Hz; ${}^3J_{\mathrm{H5-H6b}} = 2.4$ Hz; ${}^2J_{\mathrm{H6aH6b}} = -12.5$ Hz) and corresponding experimental refocused INEPT spectra.

signal intensities observed in the 'geometrical imprint domain' for $\Delta = 0.1408$ s proved to be exclusively representative for ${}^3J_{\mathrm{C(O)-O-CH}}$ contributions $\{k_i[{}^2J_{\mathrm{C(O)-CH}}] = 0$ and $k_i[{}^3J_{\mathrm{C(O)-O-CH}}] = -2.298$ and -0.8344 for the CO-1 and CO-4 systems, respectively$\}$.

Obviously, two-dimensional methods offer more advantages in terms of both efficiency and precision over conventional one-dimensional approaches. It appears exceedingly worthwhile to test the feasibility of using computed $E_{\mathrm{INEPTdec}} = f(\tau,\Delta)$ graphical representations for implementing two-dimensional correlations. Compound **4** serves as a typical example: for $\Delta = 0.1408$ s ('geometrical imprint domain,' see Fig. 5) the resulting ${}^{13}\mathrm{C}/{}^1\mathrm{H}$ 2D map leads via specific amplitude and intensity modulations to straightforward assignment of long-range ${}^nJ$ carbon atoms, according to the ${}^3J_{\mathrm{HH}}$ values of vicinal protons closed to each carbon atom involved (Fig. 6).

Another prominent feature of these 2D experiments is the possibility of the appearence/disappearence of correlations, according to characteristic Δ values. Thus, for $\Delta = 0.0254$ s the magnitude of the $k_i[{}^2J_{\mathrm{C(O)-CH}}]$ response depending on the sine term is maximum in the absence of ${}^1\mathrm{H}-{}^1\mathrm{H}$ couplings and $k_i[{}^3J_{\mathrm{C(O)-O-CH}}] \approx 0$. Conversely, for $\Delta = 0.1408$ s ('geometrical imprint domain'), $k_i[{}^3J_{\mathrm{C(O)-O-CH}}]$ is maximum and $k_i[{}^2J_{\mathrm{C(O)-CH}}] \approx 0$.

To the best of our knowledge, the proposed two-dimensional approach represents the first example of a 2D editing method based on the spatial arrangement around the atoms under consideration, rather than to the number of protons in ${}^1J$ implied.

CONCLUSION

While a specific proton-detected one dimensional ${}^1\mathrm{H}-{}^{29}\mathrm{Si}$ correlation approach has recently been envisaged for the elucidation of protective group positions in silylated vitamin D analogues (needing a complete and unambiguously prerequisite assignment of ^{1}H spectra),[13] our objective is to propose a more direct and general editing method. The present study emphasizes the significance of the influence of homonuclear coupling contributions in computed graphical representations $E_{\mathrm{INEPTdec.}} = f(\tau, \ \Delta)$ in refocused INEPT experiments. We have developed a practical, straightforward 1D and 2D spectral editing tool for the qualitative structural analysis of silylated and acetylated glycosides, taking advantage of a hitherto unknown 'geometrical imprint domain' closely linked to the spatial arrangement around the heteroatom under investigation.

The proposed method could be extended to other conformational systems.

EXPERIMENTAL

All the experiments were performed on a Bruker AC 200 spectrometer (^{1}H, 200.132 MHz; ^{29}Si, 39.761 MHz) fitted with an Aspect 3000 data system using a 10 mm broadband probe (^{29}Si 90° pulse width = 15 μ, ^{1}H 90° pulse width through the decoupler coil = 29 μs).

^{29}Si NMR spectra were recorded at room temperature as a 30% (w/v) solution in C_6D_6. Chemical

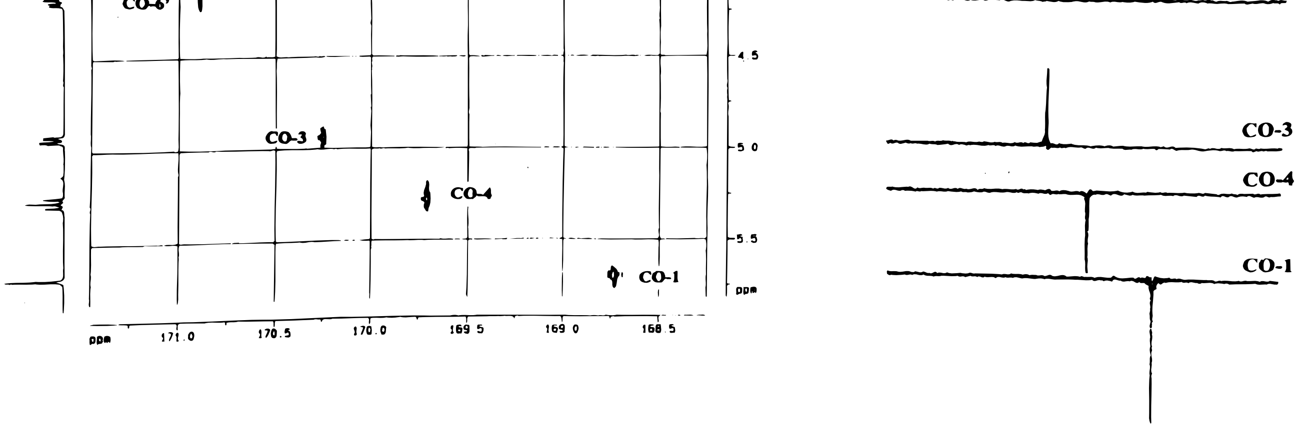


Figure 6. Contour plot of the ${}^{13}\mathrm{C}-{}^1\mathrm{H}$ 2D refocusing–decoupled INEPT correlation for $\tau = 0.0357$ s and $\Delta = 0.1408$ s recorded on 1,3,4,6-tetra-*O*-acetyl-*β*-D-mannopyranose (**4**), with corresponding slices of sp^2 carbon atoms.

shifts are referenced to TMS (internal standard) and are expressed as δ_{ppm}: methyl 2,3,4,6-tetrakis-*O*-trimethylsilyl-α-D-mannopyranoside (**1**), $Si_2 = 16.4$, $Si_3 = 17.5$, $Si_4 = 18.3$, $Si_6 = 17.3$; methyl 2,3,4,6-tetrakis-*O*-trimethylsilyl-α-D-mannopyranoside (**2**), $Si_2 = 16.4$, $Si_3 = 17.5$, $Si_4 = 18.3$, $Si_6 = 17.3$;methyl 2,3, 4,6-tetrakis-*O*-trimethylsilyl-β-D-galactopyranoside (**3**), $Si_2 = 18.7$, $Si_3 = 17.7$, $Si_4 = 16.6$, $Si_6 = 18.1$. The refocused–decoupled 1D INEPT ^{29}Si pulse sequence was performed using the standard Bruker microprogram (INEPTRD.AU). The spectrum was recorded with spectral widths of SW = 165.8 Hz or 4716.9 Hz digitized with 2K or 64K points, respectively (number scans = 32; relaxation time = 2 s).

^{13}C NMR spectra were recorded at 303 K on a Bruker (AVANCE) DPX 400 spectrometer, equipped with a digital lock and 5 mm broadband probe operating at 100.61 MHz for ^{13}C. Broadband ^{1}H decoupled ^{13}C spectra and ^{13}C INEPT spectra were recorded using the standard Bruker microprogram (^{13}C 90° PW = 7 μ, ^{1}H 90° PW = 9.5 μ) with spectral widths of SW = 903.18 Hz digitized with 16K points (number scans = 32; relaxation time = 2 s). Chemical shifts are referenced to TMS and are expressed as δ_{ppm}:1,3,4,6-tetra-*O*-acetyl-β-D-mannopyranose (**4**) CH_3 20.6, 20.7, 20.8, 20.9; $C_6 = 62.0$, $C_4 = 65.2$, $C_2 = 68.2$, $C_3 = 72.9$, $C_5 = 73.0$, $C_1 = 91.7$; $CO_1 = 168.7$, $CO_4 = 169.7$, $CO_3 = 170.2$, $CO_6 = 170.9$.

The 2D ^{13}C–^{1}H heteronuclear shift correlation Bax sequence[14] with refocusing of chemical shifts was performed in the phase-sensitive mode (TPPI) using an initial (t_1t_2) matrix of 256 × 1024 real data points, zero-filled to 1024 × 1024 points after acquisition. The spectra of (**4**) were acquired with spectral widths of 903.18 Hz in the F_2 and 1882.6 Hz in the F_1 dimension (relaxation time = 2 s; $\tau = 0.0357$ s; $\Delta = 0.1408$ s). A total of 16 transients were accumulated for the 256 increments of t_1. The data were transformed after being exponentially weighted using a line-broadening factor of 0.5 Hz over 1K points. The t_1 interferograms were multiplied by a $\pi/4$ shifted sine-bell squared function and Fourier tranformed over 1024 points.

Theoretical spectra and computed graphical $E_{\text{INEPTdec.}} = f(\tau, \Delta)$ representations were performed using a simulation program (SIMEPT),[8] available on request.

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