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# First experimental determination of two-bond $^{13}C$ isotopic effects on $^{1}H$ NMR chemical shifts

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**Abstract**

For the first time, a simple NMR methodology is proposed for the accurate determination of the effect of the substitution of $^{12}C$ by $^{13}C$ on the chemical shifts of protons separated by two-bonds in small molecules in their natural abundance.


*Keywords:* $^{13}C$ isotopic effect; 1D-TOCSY; $^{13}C$-satellites; $^{1}H$ chemical shift

The introduction of an isotopic label into a molecule produces slight changes in the chemical shifts of the neighboring nuclei that can provide relevant structural information.[1] The most common form of isotopic effect in organic molecules is the change in the $^{13}C$ NMR chemical shift observed upon deuteration of exchangeable protons, which has been exploited for assignment purposes and hydrogen bond detection.[2] Because the influence of isotopic substitution on chemical shifts depends on the mass ratio of the isotopes[3] (the isotopic effect increases with the ratio) and on the chemical shift range of the nuclei being measured[3] (the larger the range, the larger are the effects), the $^{13}C$ isotopic effect on $^{1}H$ signals is much smaller than that provided by deuteration.

While deuterium isotopic effects on $^{13}C$ signals are frequently observed over more than one-bond,[4] to the best of our knowledge only one-bond $^{13}C$ isotopic effects on $^{1}H$ signals, $^{1}\Delta H(^{13}C)$, have been reported.[5] Typical $^{1}\Delta H(^{13}C)$ values are about $-2$ ppb, where the negative sign follows the generally accepted convention that establishes that the isotopic effect is the chemical shift of a proton in the isotopomer with the heavier isotope minus that with the lighter isotope.[6] As isotopic effects decrease with the number of bonds, very small values are anticipated for two-bond $^{13}C$ effects on $^{1}H$ chemical shifts ($^{2}\Delta H(^{13}C)$), explaining why such isotopic effects have not been determined to date.

The one-bond $^{13}C$ isotopic effect on proton chemical shifts is easily noticed when analyzing the carbon satellites of a proton resonance in a $^{1}H$ spectrum because, due to the large one-bond proton–carbon coupling, the minor signal of a H*i* proton attached to a $^{13}C$*i* nucleus does not overlap with the major signal of this proton attached to a $^{12}C$*i* nucleus. The two-bond proton–carbon coupling, however, is much smaller and, as a consequence, the signals of a H*j* proton at a two-bond distance from a $^{13}C$*i* nucleus is obscured by the strong signal of the major $^{12}C$*j* isotopomer. Here, we report a convenient methodology based on the 1D-TOCSY experiment[7] to obtain the signal of the protons separated by more than one-bond from a $^{13}C$ nucleus without the presence of the disturbing signal of the predominant $^{12}C$ isotopomer. The 1D-TOCSY is a standard NMR pulse sequence that selectively excites a chosen proton resonance by a shaped pulse and transfers its magnetization to the protons that are part of the same spin system by means of a spin-lock period. The proposed

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methodology consists of the acquisition of two individual 1D-TOCSY experiments with the offset of the shaped pulse set at the frequency of the low- or the high-frequency $^{13}C$ satellites of a H*i* proton to yield 1D subspectra of the protons that belong to the same spin system as H*i* for the minor $^{13}C$ isotopomer, with the $^{13}C$ nuclei in its α (low-frequency satellite excitation) or β (high-frequency satellite excitation) states.[8]

Let us consider the outcome of such an experimental scheme for a model spin system, H1–C1–C2–H2, in which H1 is coupled to H2. The conventional 1D-TOCSY with selective excitation of H1 would give rise to a doublet corresponding to H2 of the predominant H1–$^{12}$C1–$^{12}$C2–H2 isotopomer with the same chemical shift as in the proton spectrum, whereas selective excitation of each $^{13}C$-satellite of H1 would lead to a doublet corresponding to H2 of the minor H1–$^{13}$C1–$^{12}$C2–H2 isotopomer. The H2 signals of the satellite-selective 1D-TOCSY spectra would be displaced by the two-bond $^{13}$C1–H2 coupling constant. In the absence of a two-bond $^{13}$C1 isotopic effect, H2 would have the same chemical shift in both isotopomers, implying that the H2 signal of the conventional 1D-TOCSY would be centered between the H2 signals of the satellite-selective experiments (Fig. 1A). In contrast, the existence of a two-bond $^{13}$C1 isotopic effect on H2 would prevent the H2 signals of the satellite-selective experiments from being symmetrically placed around the H2 signal of the predominant $^{12}C$ isotopomer (Fig. 1B).

Considering the small effect that we intended to measure, and knowing that the decrease of the isotopic effects with the number of bonds is less pronounced in aromatic than in aliphatic systems,[3] we chose a pyridine derivative, 2-picoline (**1**), whose proton spectrum exhibited excellent signal dispersion, to test the methodology (Scheme 1). H6 and its $^{13}C$-satellites were selectively excited in three individual 1D-TOCSY experiments to give rise to the peaks for the rest of the pyridine coupling network (Fig. 2). The superposition of the 1D-TOCSY spectra for H4 and H5 resonances is shown in Figure 3. While the H4 signal of the $^{12}C6$ isotopomer is centered between the corresponding signals of the $^{13}C6$ isotopomers, indicating the absence of an appreciable three-bond $^{13}C6$ isotopic effect, the H5

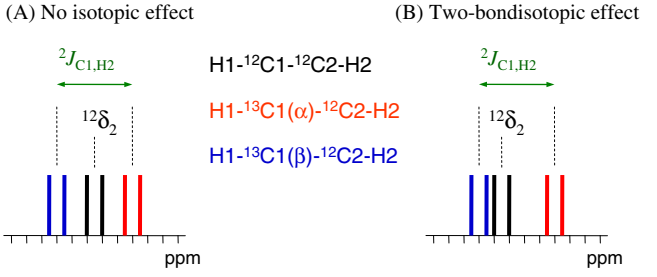
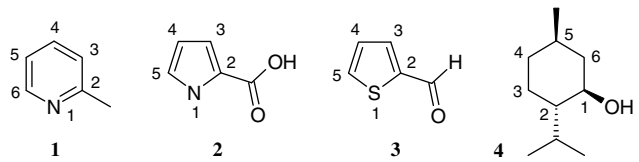



Fig. 1. Schematic representation of the signals expected for H2 in the model system H1–C1–C2–H2 in the absence (A) or in the presence (B) of a $^{12}C1/^{13}C1$ isotopic effect on the chemical shift of H2. The signals are color coded according to the isotopomer to which they correspond. A positive C1–H2 coupling is assumed.




Scheme 1. Compounds under analysis along with the atom numbering.

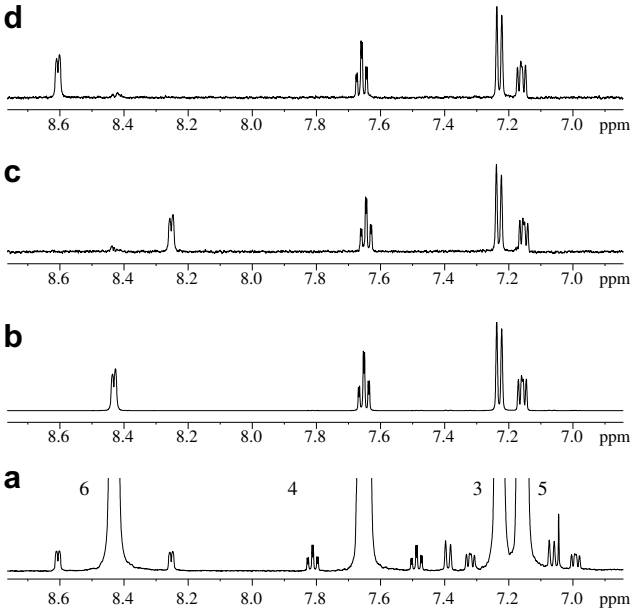



Fig. 2. 500 MHz spectra of **1** at 25 °C. (a) Conventional $^1H$ spectrum showing the low-intensity $^{13}C$-satellites; (b–d) 1D-TOCSY spectra with selective excitation of the central $^{12}C$-bonded H6 resonance (b), the low-frequency H6 satellite (c), or the high-frequency H6 satellite (d).

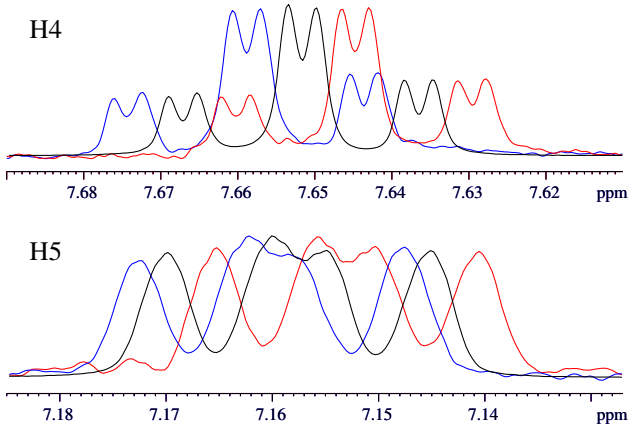



Fig. 3. Superposition of the conventional (black) and satellite selective (red and blue) 1D-TOCSY spectra for the H4 and H5 regions of **1**.

signal of the regular 1D-TOCSY is clearly closer to the H5 signal of the 1D-TOCSY in which the high-frequency $^{13}C$-satellite was excited, reflecting the existence of a non-negligible two-bond $^{13}C6$ isotopic effect on the H5 chemical shift.[9]

We also applied this methodology to two other aromatic compounds, **2** and **3**, using the proton *meta* to the heteroatom (H4) and its satellite peaks as the source of magnetization transfer. In both molecules, the superposition of 1D-TOCSY revealed that the H3 and H5 signals of the conventional 1D-TOCSY were not centered between the signals of the satellite-selective experiments, being closer to the signal arising from excitation of the high-frequency satellite. This asymmetry highlighted the existence of a two-bond $^{13}$C4 isotopic effect on the H3 and H5 chemical shifts for both heteroaromatic compounds. Furthermore, to provide an example in an aliphatic system we examined isotopic effects in menthol (**4**) by selective excitation of the H1 signal. Displacement of the signals of the $^{12}$C1 isotopomer from the center of the corresponding signals of the $^{13}$C isotopomer was observed for $H6_{eq}$ and H2 protons, located at two-bond distance from $^{13}$C1, but not for H5 and $H3_{eq}$ separated by three-bonds from this nucleus.

The magnitude and the sign of the two-bond $^{13}$C isotopic effect can therefore be measured from the displacement of a signal in the satellite-selective 1D-TOCSY spectra relative to the regular 1D-TOCSY spectrum. The exact protocol was to vary the intensity and the chemical shift of the signal of the satellite-selective spectrum for the best fit to the corresponding signal of the regular spectrum. Through this fitting procedure, depicted in Figure 4 for **3**, small isotopic shifts can be measured with excellent accuracy. The values determined for compounds **1–4** are gathered together in Table 1. As expected, the $^{2}\Delta H(^{13}C)$ isotopic effects are small, ranging between −0.6 and −1.2 ppb, and negative, that is, the $^{13}$C nucleus causes a decrease in the $^{1}$H NMR frequencies.

Table 1
Two-bond $^{13}$C isotopic effects on $^{1}$H chemical shifts in compounds **1–4**

| Compound | Carbon | Proton | Isotopic effect[a] |
|---|---|---|---|
| **1** | C6 | H5 | −1.0 |
| **2** | C4 | H3 | −0.8 |
| | | H5 | −1.0 |
| **3** | C4 | H3 | −0.9 |
| | | H5 | −1.2 |
| **4** | C1 | H2 | −0.7 |
| | | $H6_{eq}$ | −0.6 |

[a] Expressed in ppb to an accuracy of ±0.2 ppb.

The major advantage of the proposed methodology is that carbon decoupling during acquisition is not required, avoiding temperature variations and distorsion of resonance lineshapes arising from the radiative effects of carbon decoupling. Because the isotopic effects that we intended to measure are very small, the deleterious effect of carbon decoupling may cause a shift comparable to the isotopic shift, affecting the reliability of the measurement.[5b] Remarkably, isotopic shifts less than the linewidth can be easily measured from the signal displacement in highly-resolved 1D spectra. In contrast to other studies of isotopic substitution that often require extensive synthetic efforts, our methodology takes advantage of the 1% random distribution of $^{13}$C found in nature and no labeling is necessary. On the other hand, because the proposed approach involves selective excitation of the $^{13}$C-bonded

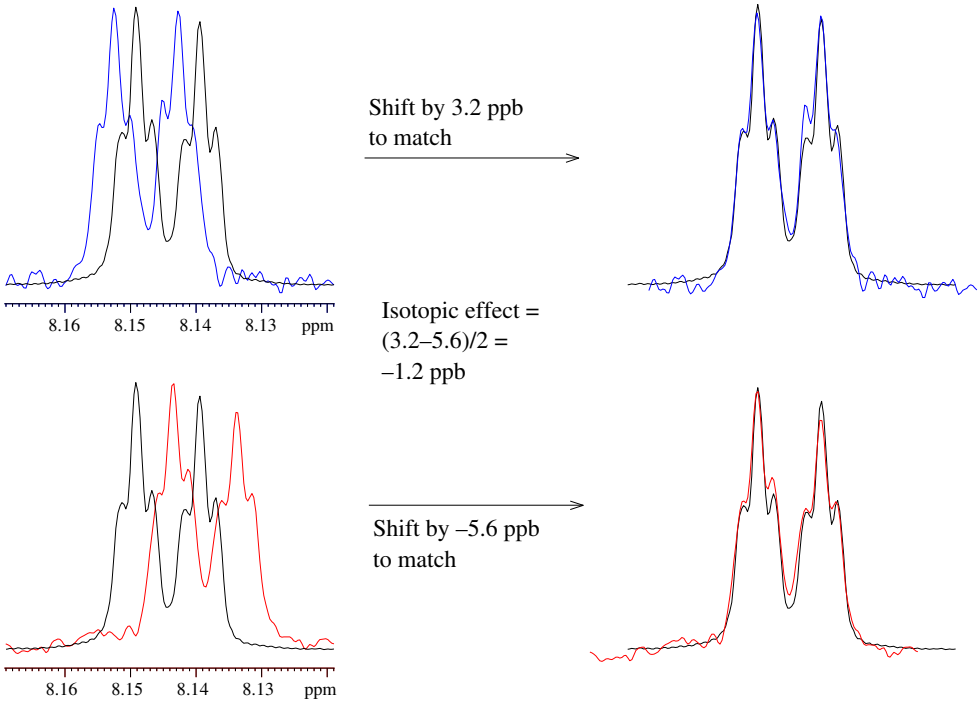



Fig. 4. Schematic representation of the fitting protocol to measure two-bond isotopic effects in **3**: (a) conventional (black) and high-frequency satellite-selective (red) 1D-TOCSY spectra; (b) conventional (black) and low-frequency satellite-selective (red) 1D-TOCSY spectra.

satellites without affecting $^{12}$C-bonded resonances, one limitation of the methodology is that it can only be applied to signals that are far enough apart from other signals in the $^{1}$H spectrum. In addition, due to its reliance on magnetization transfer via proton–proton couplings, it is not suited to the determination of isotopic effects caused by quaternary carbons.

In summary, we have devised a successful methodology for the determination of two-bond $^{13}$C isotopic effects on proton chemical shifts around $-0.6/-1.2$ ppb in non-labeled organic molecules, as exemplified by **1**–**4**. The measured values are small, although well above experimental uncertainties, and negative, indicating that the substitution of a $^{12}$C by a $^{13}$C isotope causes a detectable shielding on the protons separated by two-bonds. In addition, it can be confirmed that these effects are practically negligible at a separation of three-bonds. It is noteworthy to mention that the data collected in this manuscript may be of significant interest as a stringent test of ab initio calculations of proton chemical shifts because isotopic shifts are directly related to the shielding surface of the resonant nucleus.[10]

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9. Compounds **1**–**4** were purchased from a commercial source and used without further purification. The compounds (ca. 15 mg) were dissolved in DMSO (**1**–**3**) and $CDCl_3$ (**4**) and transferred into an NMR tube. The NMR experiments were carried out using a 500 MHz spectrometer at 25 °C equipped with a 5 mm, inverse, broadband probe head with a *z*-gradient coil. The number of transients of the 1D-TOCSY experiments was 8 when the $^{12}$C-bonded signal was inverted and 128 when a $^{13}$C-satellite was inverted. The digital resolution of the 1D-TOCSY spectra was 0.08 Hz/pt. The 1D-TOCSY pulse sequence was comprised of an initial hard 90° $^{1}$H pulse to excite all the proton resonances followed by the [gradient—selective 180° $^{1}$H pulse—gradient] sandwich that retained the magnetization of the chosen proton, which was then transferred to the protons of the same spin system by using a DIPSI-2 mixing scheme. The length of the selective $^{1}$H pulse (1% truncated Gaussian shape) was 60 ms to achieve the desired selectivity. A *z*-filter element consisting of a simultaneous swept-frequency 180° pulse and a gradient was introduced before the DIPSI-2 block to eliminate zero-quantum interference (Thrippleton, M.J.; Keeler, J. *Angew. Chem., Int. Ed.* **2003**, *42*, 3938). The mixing time of the DIPSI-2 block was optimized in each compound for maximum signal intensity.
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