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Enantiodifferentiation through Frequency-Selective Pure-Shift ^{1}H Nuclear Magnetic Resonance Spectroscopy

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A frequency-selective 1D ^{1}H nuclear magnetic resonance (NMR) experiment for the fast and sensitive determination of chemical-shift differences between overlapped resonances is proposed. The resulting fully homodecoupled ^{1}H NMR resonances appear as resolved 1D singlets without their typical *J*(HH) coupling constant multiplet structures. The high signal dispersion that is achieved is then exploited in enantiodiscrimination studies by using chiral solvating agents.

Nuclear magnetic resonance (NMR) spectroscopy in the presence of chiral auxiliaries is a particularly well-adapted technique for determining the enantiomeric purity and, in some cases, the absolute configuration of chiral molecules.[1] Different approaches are available to accomplish enantiodifferentiation, including chemical derivatization,[2] chiral solvating agents (CSAs),[3] and the use of chiral liquid crystals.[4] In the case of CSAs, the NMR method simply requires the use of a suitable chiral derivative that converts the initial indistinguishable mixture of enantiomers into a chemical-shift (δ)-resolved mixture of complementary diastereomeric complexes. As soon as there is a large enough δ nonequivalence to achieve resolution between the signals ($\Delta\Delta\delta$) of analogous nuclei in these diastereomeric complexes, integration can enable the direct measurement of enantiomeric purity. However, homonuclear scalar couplings (J(HH)) broaden ^{1}H NMR resonances, and accurate enantiomeric excess (*ee*) quantification by optimum signal discrimination is often hampered because of partial signal overlapping and low chemical-shift dispersion ($\Delta\Delta\delta \ll \Delta\omega$, where $\Delta\omega$ is the overall width of the multiplet). The use of selective homonuclear decoupling to simplify the multiplet structure is insufficient to completely resolve overlapping.[5] However, the analysis of better-resolved fully decoupled singlet resonances in heteronuclear-decoupled ^{13}C NMR spectra is an alternative, which avoids signal overlapping, but its low sensitivity remains a limiting factor for practical use.[6] Recently, several NMR methods have been proposed to obtain pure chemical-shift ^{1}H NMR spectra.[7–11] Based on a recent instant broadband homodecoupled experiment,[10] an analogous region-selective version that does not suffer sensitivity loss but maintains the benefits of obtaining simplified singlet resonances has been reported.[11] We evaluate here the potential of this strategy for the fast and efficient enantiodifferentiation of organic molecules using CSAs.

The proposed NMR experiment (Figure 1) can be understood as a homodecoupled version of the regular 1D single pulsed-field-gradient echo (SPFGE) scheme, in which a frequency-selective 180° pulse is applied to ^{1}H NMR resonances of interest; the novelty lies in the incorporation of a broadband homodecoupling element into the acquisition period.[11] The resulting 1D ^{1}H NMR spectrum only shows the selected resonances as collapsed singlet lines, without their typical *J*(HH) multiplet structure, and from which accurate chemical-shift values can be determined, even for overlapped resonances. As the sensitivity is fully retained, data acquisition can be performed quickly with the same spectrometer time required for a conventional ^{1}H NMR spectrum. Experimentally, only a single selective 180° pulse needs to be setup, as a function of its excitation offset and the required selectivity for both excitation/homodecoupling purposes. We found that Gaussian-shaped pulses with a duration of around 10–20 ms provide good results, in terms of resolution, without a considerable decrease in the signal-to-noise ratio (SNR), owing to transverse relaxation during acquisition. Average line widths at half height of the singlets ($\nu_{1/2}$) of about 3.5–4.0 Hz are achieved by using homodecoupling settings of $\Delta = 15$–25 ms, $n = 11$–20, and $AQ = 600$ ms (where AQ is the acquisition time and n the number of concatenated loops), whereas $\nu_{1/2} = 2.3$–2.7 Hz values are generally found in

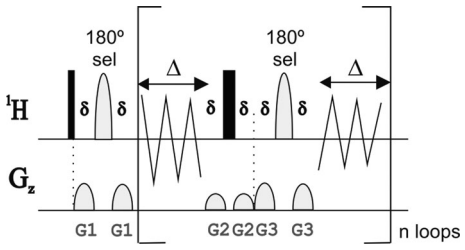

Figure 1. Pulse sequence for obtaining fully homodecoupled singlet resonances in a selected narrow part of the ^{1}H NMR spectrum. Broadband homodecoupling during detection was achieved by applying a pair of hard/selective 180° pulses (represented as solid and shaded shapes) at the middle of $2\Delta = AQ/n$ periods. Gradients G1, G2, and G3 flanking the refocusing pulses are individually optimized to provide a clean spectrum. δ represents the duration of a pulsed field gradient and its recovery delay.

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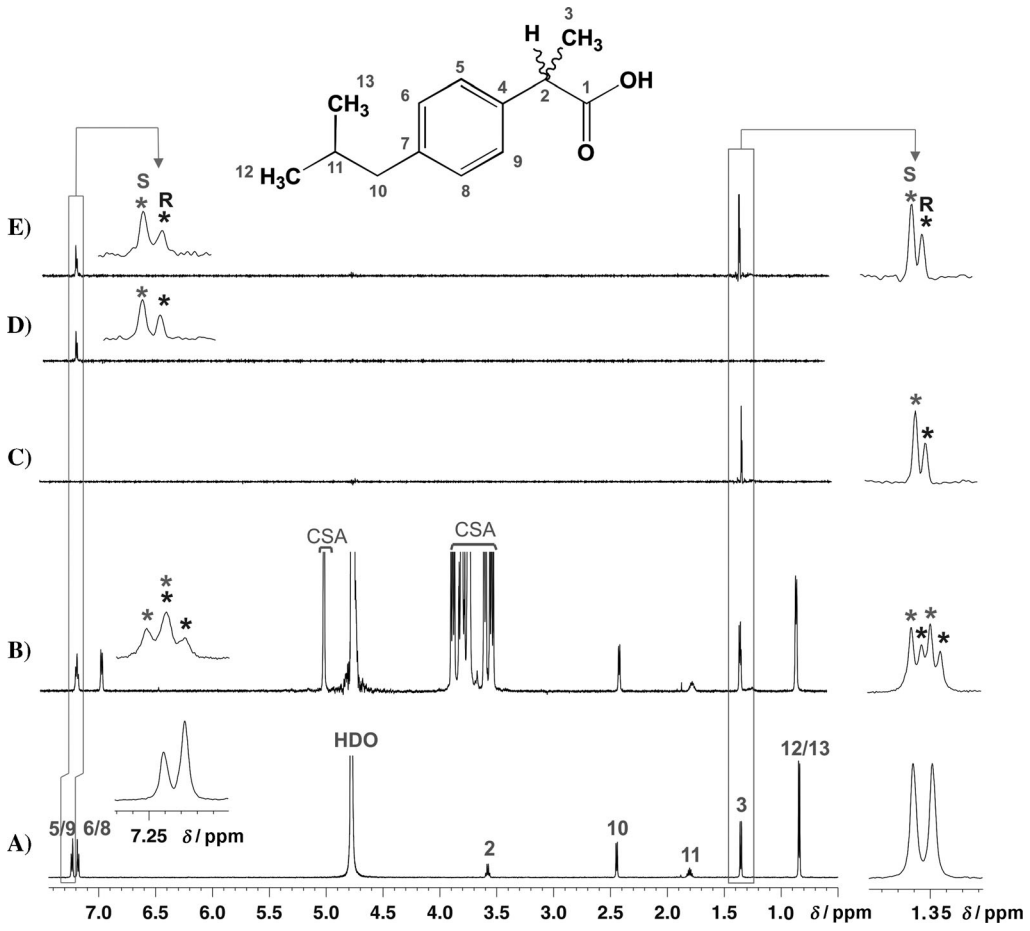

Figure 2. 600 MHz ^{1}H NMR spectra of 2.8 mM (*R*,*S*)-ibuprofen (35:65 proportion) in D_2O: A) before and B) after the addition of 3.6 equivalents of β-CD as the CSA. Selective pure-shift ^{1}H NMR spectra acquired according to Figure 1 after selection of the C) H3, D) H5, and E) both H3 and H5 protons with a Gaussian-shaped 180° pulse of 20 ms ($\Delta = 25.8$ ms, $AQ = 568$ ms, and $n = 11$). For comparison, all spectra were acquired and processed under the same conditions (a single scan for each individual 1D spectrum has been recorded with the same receiver gain) and plotted in the same vertical scale to visualize real absolute sensitivities.

the regular ^{1}H NMR spectrum (see Figure S1 in the Supporting Information).

As a proof of principle, the practicality of the method is demonstrated in the study of an (*R*,*S*) mixture of ibuprofen in the presence of β-cyclodextrin (β-CD) as the CSA (Figure 2).[3d] Whereas the conventional ^{1}H NMR spectrum shows poor signal separation between equivalent diastereomeric protons (Figure 2B), the clean homodecoupled 1D spectra simplifies the appearance of complex peaks and shows separated singlet resonances, which facilitates a better analysis and quantification (Figure 2C,D). It is worth noting that the sensitivity for each individual selective homodecoupled 1D spectrum is keep at a similar level to the conventional ^{1}H NMR spectrum, and, therefore, each one of these spectra can be obtained by using a single scan within few seconds and without any extra data processing requirement.

Figure 3 shows another example of the fast and sensitive discrimination of several ^{1}H NMR resonances belonging to a racemic mixture of (*R*,*S*)-1-aminoindan in the presence of Pirkle alcohol as the CSA.[3c] A straightforward comparison between the conventional (Figure 3B) and the fully homodecoupled multiplets (Figure 3C) shows that a simpler and more reliable determination of the chemical-shift differences and *R*/*S* molar ratios is possible, considering the highly dispersed singlets that are independent of the original multiplet complexity. In terms of quantification, it is important to note that deviations of the homodecoupling conditions ($\Delta \ll 1/J_{HH}$)[10] can lead to sidebands flanking each pure-shifted resonance at a spacing of $2n/AQ$ (see Tables S1 and S2 in the Supporting Information).

Although one limitation of the method could be its frequency-selective nature, it is not restricted to a single resonance for each individual experiment, because multiple signals can be simultaneously monitored by using band-selective[11] or multiple-frequency pulses,[12] as long as the excited protons are not mutually *J*-coupled (Figure 2E). The proposed method surpasses some other NMR approaches to discriminate enantiomers because it avoids time-consuming 2D acquisitions and/or measurements made from the unresolved indirect dimension.[13] However, homodecoupled ^{1}H NMR signals for all available resonances in the spectrum can be obtained by using other broadband pure chemical-shift NMR methods[7,11–13] although they can suffer significant decreases in sensitivity. The

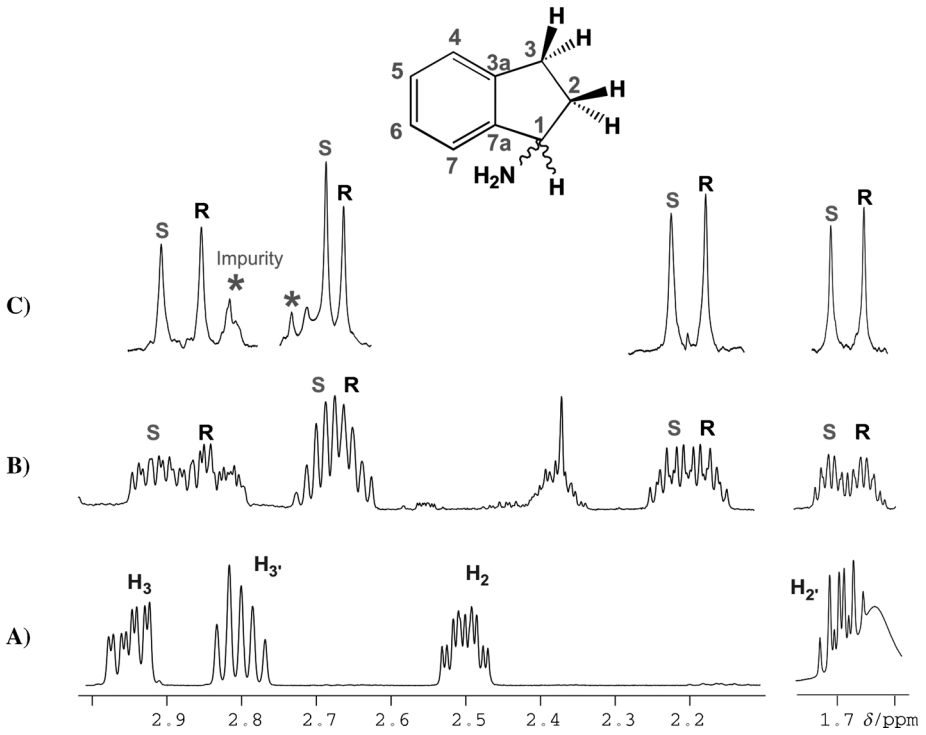

Figure 3. 600 MHz ^{1}H NMR spectra of 50 mM (*R,S*)-1-aminoindan (1:1 proportion) in $CDCl_3$: A) before and B) after the addition of 4.5 equivalents of (*R*)-(−)-1-(9-anthryl)-2,2,2-trifluoroethanol (Pirkle alcohol) as the CSA. C) Expanded multiplets extracted from individual selective 1D homodecoupled experiments acquired according to Figure 1 by using a Gaussian-shaped 180° pulse of 20 ms ($\Delta = 18.93$ ms, $AQ = 2.27$ s, and $n = 60$). For comparison, all spectra were acquired and processed under the same conditions (a single scan for each individual 1D spectrum has been recorded with the same receiver gain) and plotted in the same vertical scale to visualize real absolute sensitivities.

projection along the detected dimension of a *J*-resolved experiment requires a 2D-acquisition mode, and, therefore, the SNR reduction is proportional to the number of acquired increments.[7] Otherwise, the original Zangger–Sterk (ZS) method shows better line widths, but it requires a pseudo-2D data-collection process and presents severe sensitivity losses, owing to spatial frequency encoding.[8] Recently, single-shot ZS methods have been proposed for the fast acquisition of broadband homodecoupled 1D ^{1}H NMR spectra, but they also experience considerable sensitivity losses because of ^{13}C editing[9] or spatial selection.[10] The use of multiple slice selection through sequential or simultaneous slice excitation[14] can improve the relative SNR, but the sensitivity levels are still far from those obtained in the conventional ^{1}H NMR spectra. In terms of SNR per time unit, a single selective method is more than one order of magnitude more sensitive than the aforementioned pure-shift methods, which ensures that, for small molecules, recording series of individual selective 1D experiments can be faster and more effective than running a broadband experiment. As an example, the experimental SNR of each selective experiment is about 20 times higher than the real-time instant ZS experiment.[10] A comparison on the relative SNR for several pure-shifts methods can be found in Figure S2 (see the Supporting Information).

Interestingly, the proposed homodecoupled 1D method can be extended for the rapid visualization of singlet signals for those resonances that appear in highly overcrowded areas and that, in many cases, cannot be directly observed. This is the case for the H2 proton of ibuprofen, which resonates just below the large signals belonging to the CSA in the conventional ^{1}H NMR spectrum (Figure 4B). This hidden signal can quickly become observable by using a sensitive total correlation spectroscopy (TOCSY) transfer from another isolated proton resonance (Figure 4C).[15] Thus, a homodecoupled version of the selective TOCSY experiment can be designed by incorporating the detection period, described in Figure 1, into the conventional experiment (see Figure S3 in the Supporting Information). The two simplified singlets, corresponding to the H2 proton in *R* and *S* derivatives, can rapidly be distinguished, resolved, and quantified ($\Delta\Delta\delta = 10.44$ Hz) with enhanced sensitivity and without CSA signal interference (Figure 4C,D).

In summary, we have demonstrated that the homodecoupled SPFGE method is a robust and sensitive analytical NMR spectroscopy tool for the fast and simple discrimination of chemical-shift differences in overlapped signals and for the determination of the *ee* in the presence of CSAs. Its major advantage lies in the single-scan and 1D acquisition modes, as the resulting simplified singlet signals facilitate a better analysis. It has been shown that homodecoupled signals can also be retrieved for resonances obscured by other more intense signals or in overcrowded regions by using a preparatory TOCSY editing. Much work is in progress to use these powerful pure-shift methodologies for solving other common problems caused by NMR signal overlapping.

Experimental Section

All NMR experiments were performed by using a 600 MHz BRUKER Avance-III spectrometer equipped with a TXI probe. Complete experimental details, a comparison of the experimental sensitivity of several pure-shift NMR experiments, a description of the homodecoupled selective TOCSY pulse scheme, and a table showing the measured $\Delta\Delta\delta$ and *R*/*S* molar ratio values measured by signal integration and line fitting can be found in the Supporting Information.

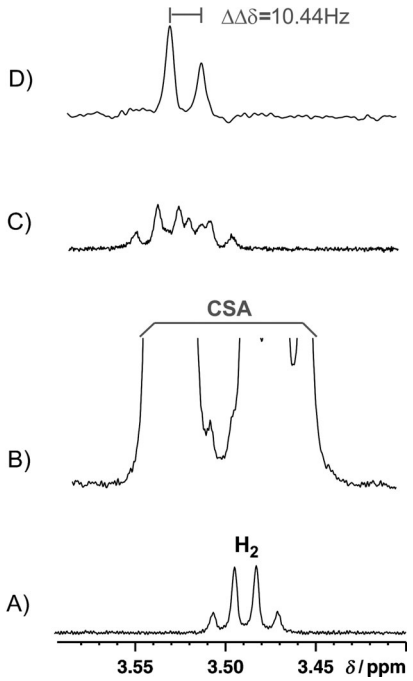

Figure 4. A, B) Expanded region corresponding to the ^{1}H NMR spectra of Figure 2A and 2B, respectively. C) Conventional and D) homodecoupled 1D TOCSY spectra showing the H2 proton after initial selective excitation of the H3 proton followed by a 60 ms TOCSY transfer. Gaussian-shaped 180° pulses of 20 ms were used for both excitation (on H3 protons at $\delta = 1.35$ ppm) and homodecoupling (on H2 protons). Spectra B–D) were acquired (four scans each one, with the same receiver gain), processed and plotted under the same conditions (see the Supporting Information) to visualize real absolute sensitivities.

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