Enantiodifferentiation through frequency-selective pure shift ^{1}H NMR

SeRMN

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Introduction

NMR-aided discrimination of enantiomers using chiral solvating agents (CSAs) is a well established method to carry out enantiodifferentiation studies. The analysis is traditionally performed by observing chemical shift differences ($\Delta\Delta\delta$) in ^{1}H signals by conventional 1D ^{1}H NMR spectra. However signals overlapping caused by low $\Delta\Delta\delta$ values and homonuclear scalar couplings (J_{HH}) lead to the lack of spectral signal dispersion that preclude a straightforward analysis.

Recently, pure shift NMR spectroscopy has emerged as a promising tool to simplify the typical J_{HH} multiplicity pattern of ^{1}H signals to singlets.[1-3] This affords a general improvement on signal dispersion that allows an improved analysis of complex and overcrowded resonances. Based on the full-sensitive ^{1}H-HOBS experiment,[3] a frequency-selective technique for the fast and simple discrimination of $\Delta\Delta\delta$ in overlapped signals and for the determination of the R/S molar ratio in the presence of CSAs is here proposed.[4]

Methodology

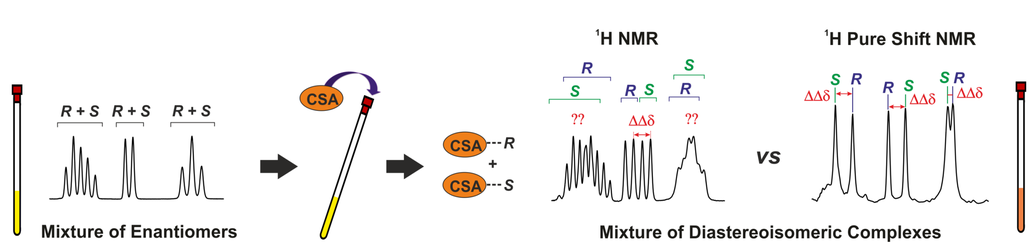

Figure 1. The CSA converts the initial indistinguishable mixture of enantiomers into a chemical-shift (δ)-resolved mixture of complementary diastereoisomeric complexes. When $\Delta\Delta\delta$ between the signals of analogous nuclei in these diastereoisomeric complexes is large enough, the integration can enable the direct measurement of enantiomeric purity. However, if $\Delta\Delta\delta$ is lower than the overall width of the multiplet, signals overlapping hamper the accurate measurement. To solve signal overlap problems we propose the use of band/frequency homodecoupled ^{1}H NMR experiment which affords pure shift spectra in particular areas of the ^{1}H spectrum where do not appear mutually J_{HH}-coupled protons.

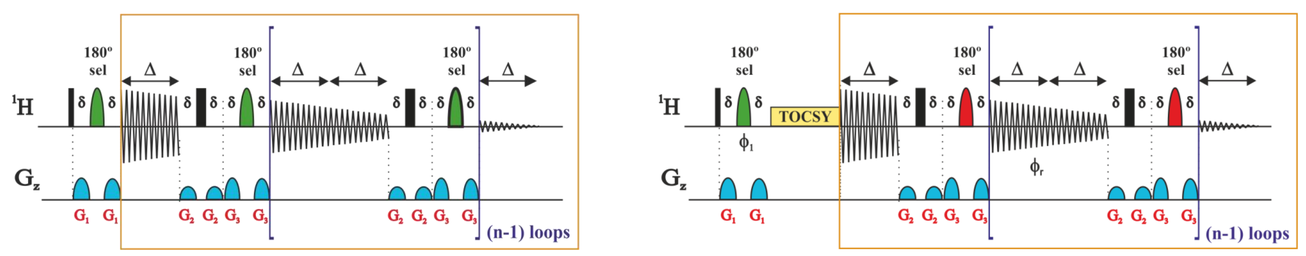

Figure 2. A) Pulse sequence for obtaining fully homodecoupled singlet resonances in a selected narrow part of the ^{1}H NMR spectrum. Broadband homodecoupling during detection was achieved by applying a pair of hard/selective 180º pulses at the middle of 2Δ=AQ/n periods. Gradients G_1, G_2, and G_3 flanking the refocusing pulses are individually optimized to provide a clean spectrum. δ represents the duration of a pulsed field gradient and its recovery delay. B) Pulse scheme for the selective and homodecoupled 1D TOCSY experiment. A minimum four-step phase cycle is used: ϕ_1=x,y,-x,-y and ϕ_r=x,-x. In contrast to the original homodecoupled scheme (A), the features of the two selective 180º pulses are here different: the first pulse (green) is applied to an isolated resonance whereas the pulse applied during the detection period (red) is applied to a relayed resonance.

Experimental Part

The practicality of the method is demonstrated in the study of two samples: (**1**) a racemic mixture 50mM (*R,S*)-1-aminoindan in $CDCl_3$ in the presence of 4,5 equivalents of Pirkle alcohol as CSA and (**2**) a mixture of 2.8mM (*R,S*)-ibuprofen (35:65 proportion) in D_2O in the presence of 3.6 equivalents of β-cyclodextrin as CSA. A straightforward comparison between the conventional (Fig. 3B) and the fully homodecoupled multiplets (Fig. 3C) shows that a simpler and more reliable determination of $\Delta\Delta\delta$ and R/S molar ratio is possible. This method is not restricted to a single resonance for each individual experiment, multiple signals can be simultaneously monitored by using multiple-frequency pulses, as long as the excited protons are not coupled (Fig. 5E). Homodecoupled ^{1}H NMR signals for all available resonances in the spectrum can be obtained by using other broadband pure shift NMR methods[1,2] although they suffer a significant decreases in sensitivity (Fig. 4) . It also has been shown that homodecoupled signals can also be retrieved for resonances hidden by other more intense signals or in overcrowded regions by using a preparatory TOCSY editing (Fig. 6).

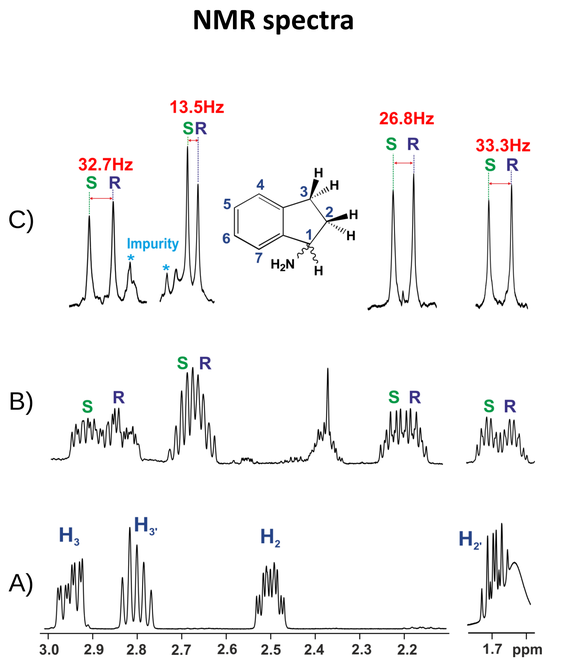

Figure 3. 600 MHz ^{1}H NMR spectra of **1** : A) before and B) after the addition of CSA. C) Expanded multiplets extracted from individual selective 1D homodecoupled experiments acquired according to Fig. 2A by using 20ms 180º Gaussian pulse (Δ=18.93ms, AQ=2.27s and n=60). All spectra were acquired with a **single scan** and plotted in the same vertical scale to visualize the real sensitivities.

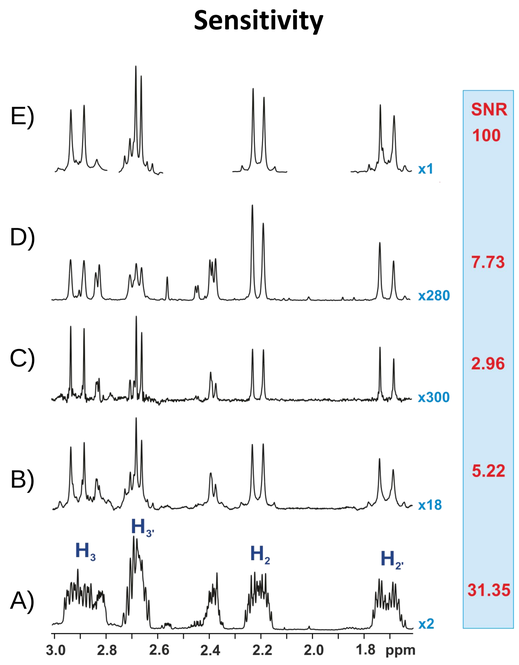

Figure 4. Comparison of signal to noise ratio (SNR) per time unit obtained for several pure shift experiments of **1**. A) ^{1}H NMR; B) instant Zangger-Sterk (ZS)[2c]; C) pseudo-2D ZS[2b]; D) internal projection of 2D J-resolved and E) multiplets extracted from individual selective 1D homodecoupled experiments. All experiments were recorded with the same experimental time (5min).

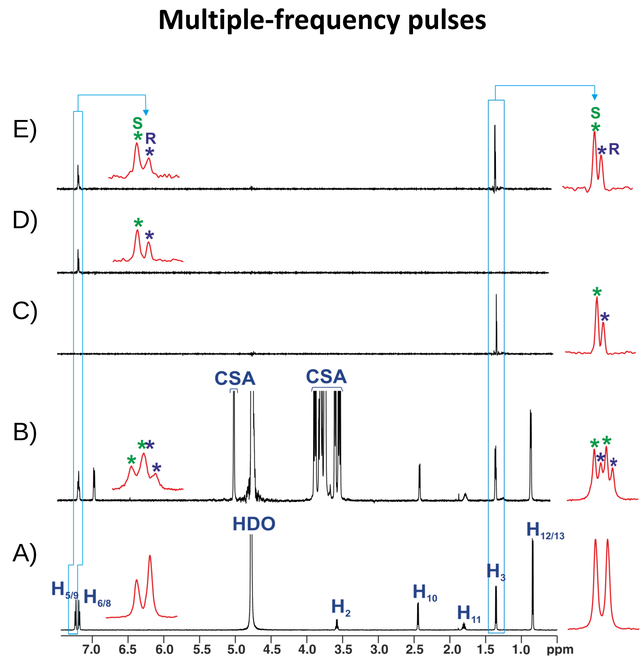

Figure 5. 600 MHz ^{1}H NMR spectra of **2**: A) before and B) after the addition of CSA. Selective pure shift ^{1}H NMR spectra acquired according to Fig. 2A after selection of the C) H_3, D) H_5, and E) both H_3 and H_5 protons with a 20ms Gaussian-shaped 180º pulse (Δ=25.8ms, AQ=568ms, and n=11). All spectra were acquired and processed under the same conditions and plotted in the same vertical scale to visualize real absolute sensitivities.

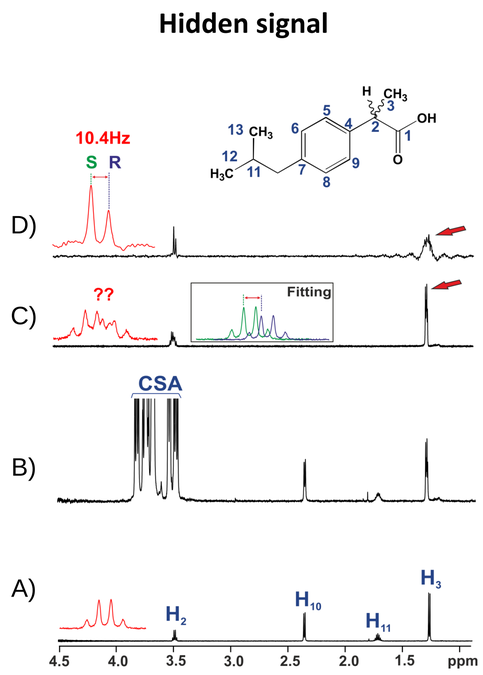

Figure 6: ^{1}H NMR spectra of **2**: A) before and B) after the addition of CSA; C) selective 1D TOCSY spectrum after selection of H_3; D) homodecoupled selective 1D TOCSY (mixing time of 60ms) spectrum acquired using the scheme of Fig. 2B. 20ms Gaussian pulse was used to excite H_3 and homodecoupled H_2 during the acquisition period (Δ=14.2ms, n=20, AQ=568ms).

Summary & Conclusions

- ✓ The frequency-selective homodecoupled 1D ^{1}H NMR experiment is a fast and efficient tool for enantiodifferentiation studies by using CSAs.
- ✓ This pure shift method simplifies the multiplets to singlets signals facilitating a better analysis.
- ✓ The discrimination of $\Delta\Delta\delta$ and the determination of R/S molar ratio are fast and simple.
- ✓ Data acquisition can be performed quickly (single scan and 1D homodecoupled acquisition mode) retaining full sensitivity.

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