Ultra-high-resolved NMR: Analysis of complex mixtures of compounds with near-identical ^{1}H and ^{13}C NMR spectra

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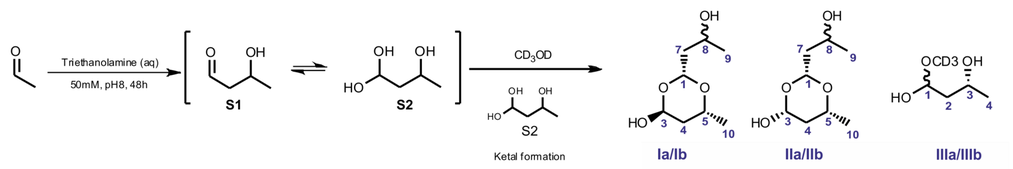
Introduction

NMR spectroscopy is the most powerful analytical tool to characterize the structure of molecules in solution. A high spectral resolution is mandatory for identifying individual resonances and to perform accurate measurements of chemical shifts or coupling constant. Over the years, NMR has demonstrated its tremendous capacity to analyze complex mixtures of compounds, where a large number of overlapped signals can be present. However, direct analysis is often limited by the lack of appropriate signal dispersion due to small chemical shift differences ($\Delta\delta$) and to the wide J_{HH} coupling patterns. A successful characterization can be further complicated when trying to differentiate structural compounds exhibiting extremely small $\Delta\delta$ and similar *J*-coupling patterns between analogous protons, resulting of a superposition of near-identical NMR spectra.

In this work we present a simple strategy for obtaining ultra-high-resolved NMR spectra that greatly facilitates the analysis of highly congested spectral regions.[1] This strategy is based on the combination of several resolution-enhanced techniques such as pure shift,[2] non-uniform sampling (NUS)[3] and spectral aliasing techniques (SA)[4] into a single NMR experiment.

Results

The power of the proposed method is illustrated in the analysis of a challenging real sample consisting of a mixture of several unknown compounds with near-identical ^{1}H and ^{13}C NMR spectra that were finally determined as three pairs of diasteroisomeric derivates (Scheme 1).

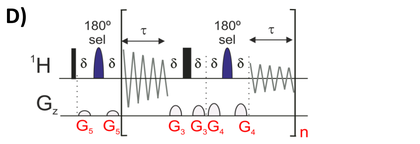


Scheme 1: Formation, structure and numbering of the three pairs of diasteroisomer compounds present in the analyzed mixture.

High-resolved 1D NMR Spectra

At first glance, the analysis of conventional ^{1}H NMR (Figure 1A) and homo- and heteronuclear 2D NMR spectra of the mixture does not provide enough information to carry out the complete characterization, mainly due to the lack of sufficient digital and signal resolution.

Recently, a new HOmodecoupled Band-Selective (HOBS) NMR method[5] has been proposed to collect pure shift NMR spectra of specific regions without sacrificing sensitivity (Figure 1D). The presence of multiple components was established from the full sensitivity pure shift ^{1}H HOBS[5] (Figure 1B,C) and the standard $^{13}C\{^{1}H\}$ (Figure 3) spectra. Most of the ^{1}H and ^{13}C signals appear doubled and the fast distinction of extremely small $\Delta\delta$ confirm the presence of very similar compounds.



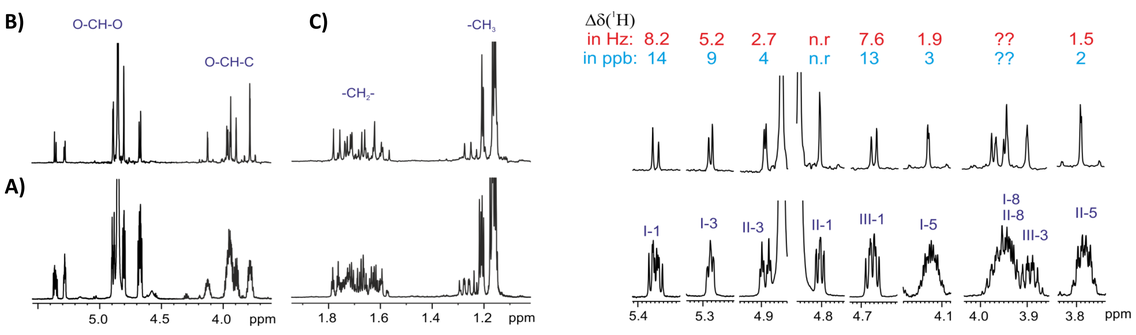


Figure 2: 600 MHz A) conventional and B,C) HOBS ^{1}H NMR spectra of the mixture in CD_3OD. Two separate 1D HOBS spectra were collect in region around (B) δ 3.5-5.5 ppm and (C) δ 1.0-1.8 ppm using in both cases a 2.5 ms REBURP 180º pulse (t_{exp}=30 s). On the right, the expanded ^{1}H multiplets resonating around δ 3.5-5.5 ppm show $\Delta\delta$ (in Hz and ppb) observed for analog protons in the mixture of the diasteroisomes. D) Pulse sequence of the HOBS 1D experiment. Rea- time homodecoupling during acquisition is achieved by combination of a hard and band-selective 180º ^{1}H pulse at the middle of 2τ=AQ/n periods.

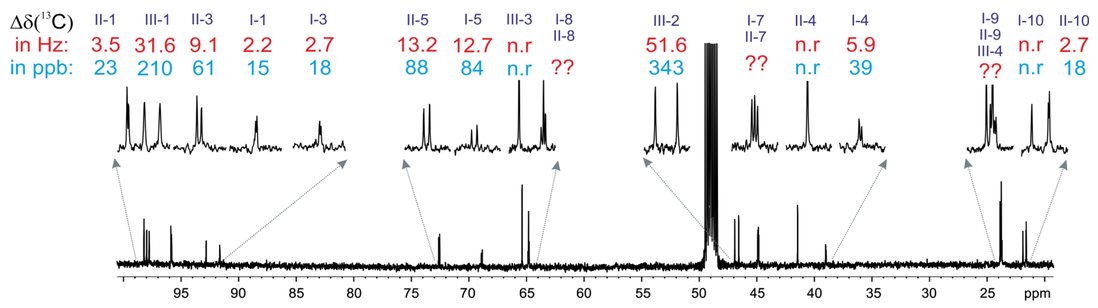
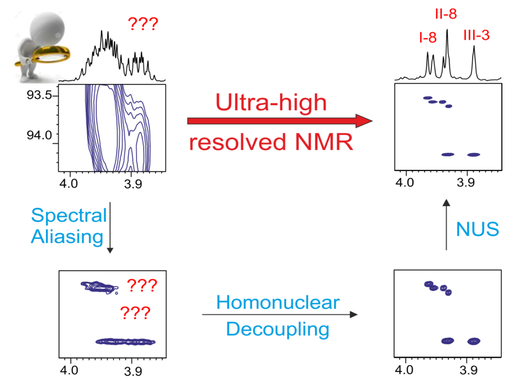


Figure 3: 150.62 MHz 1D $^{13}C\{^{1}H\}$ NMR spectrum of the mixture in CD_3OD. Expanded areas show the chemical $\Delta\delta$ (in Hz and ppb) observed for analog carbons in the mixture of the diasteroisomes.

Despite the high resolution in 1D ^{1}H HOBS and ^{13}C NMR spectra (about 1.5 and 2 Hz, respectively) some signals are not differentiated due to $\Delta\delta$ is extremely small. In other cases, the presence of multiple peaks in a narrow range of frequencies prevents the unambiguous identification of pair diasteroisomeric resonances and therefore the determination and assignment of $\delta(^{1}H)$ and $\delta(^{13}C)$.

Ultra-high-resolved 2D NMR Spectra



Scheme 2: Schematic illustration of the resolution enhancements achieved in both dimensions after combining spectral aliasing, broadband homodecoupling and NUS into a single high-resolution HSQC experiment.

A novel NMR strategy to perform a complete and fast ^{1}H and ^{13}C chemical shift assignment is here proposed. It consists on combining several resolution-enhanced techniques into the same experiment to obtain ultra-high-resolved 2D NMR spectra in conventional acquisition times (Scheme 2). The signal resolution along the ^{1}H dimension is improved by the implementation of the full sensitivity homonuclear decoupling HOBS methodology.[4] Additionally, the digital resolution along the ^{13}C dimension is improved by using a reduced ^{13}C spectral width of a few ppm (spectral aliasing),[3] optionally combined with NUS methodology.[4]

In this work a suite of modern ultra-high-resolved HOBS pulse schemes (Figure 3) have been applied to obtain fully homodecoupled HOBS-HSQC[5a] (Figure 4), HOBS-HSQC-TOCSY[1] (Figure 5), and HOBS-HSQMBC[6] (Figure 6) spectra for a set of non-mutually *J*-coupled protons resonating in a selected region of the ^{1}H dimension. Using this approach, $\Delta\delta$ of 1 and 5 ppb for ^{1}H and ^{13}C can be differentiated, respectively.

NMR Pulse sequences

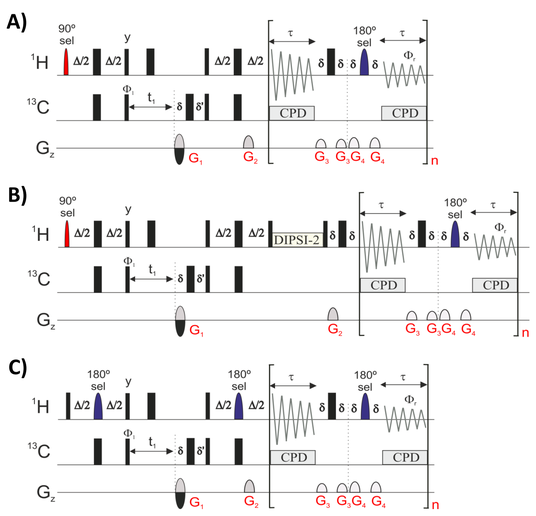


Figure 3: NMR pulse sequence of A) HOBS-HSQC, B) HOBS-HSQC-TOCSY, C) HOBS-HSQMBC experiments. The basic phase cycling is Φ_1=x,-x and Φ_r=x,-x. δ is the duration of gradients and the recovery delay. The INEPT delays are set to $\Delta = 1/(2.^{1}J_{CH})$ in HSQC and HSQC-TOCSY experiment and to $\Delta+p180=1/(2.^{n}J_{CH})$ in selHSQMBC experiment. The gradient ratio G_1:G_2 was set to 80:20.1 (percentage of the maximum strength of 53.5 G/cm).

SA-HSQC *vs* SA-HOBS-HSQC

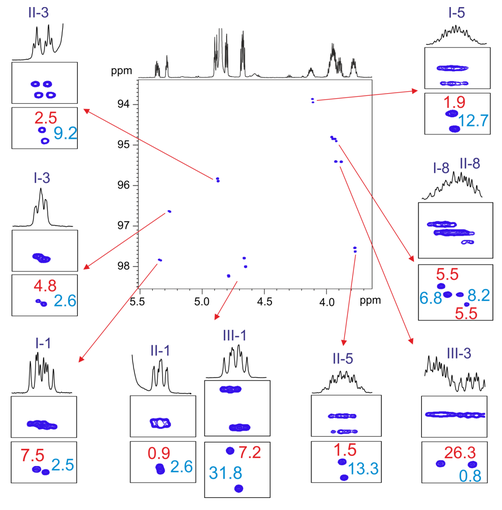
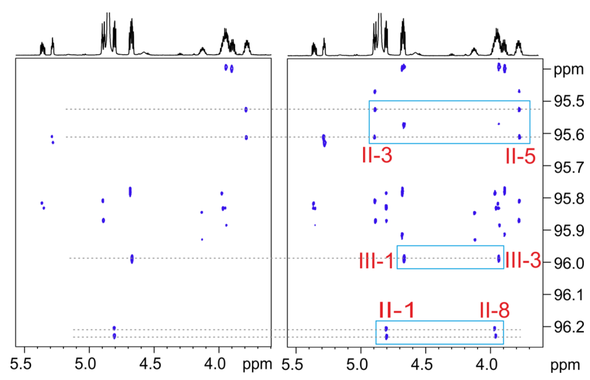


Figure 4: 2D HOBS-HSQC spectrum of region around δ 3.5-5.5 ppm acquired with a SW(^{13}C)=5 ppm and with 50% NUS. The 90º and 180º band-selective pulses were a 1,75 ms EBURP and 2,5 ms REBURP, respectively. For homodecoupling, 130 loops (n) were concatenated with τ=AQ/2=9 ms (t_{exp}=14 min). G3, G4 and G5 were set to 21.9, 33.7 and 12.3 G/cm, respectively. Expanded 2D cross-peaks corresponding to the (top) SA-HSQC and (bottom) SA-HOBS-HSQC spectra are shown for comparison. Experimental $\Delta\delta(^{1}H)$ and $\Delta\delta(^{13}C)$ are expressed in Hz.

SA-HOBS-HSQC *vs* SA-HOBS-HSQC-TOCSY



SA-HOBS-HSQMBC

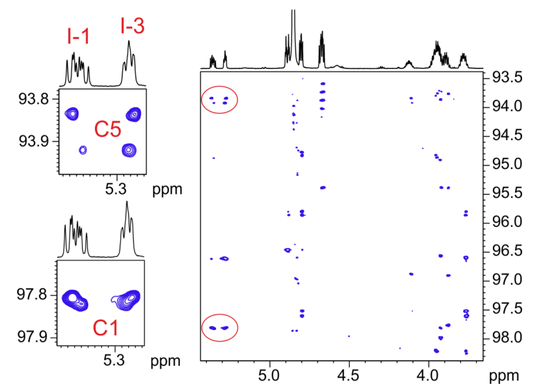


Figure 5: Comparison of SA-HOBS-HSQC (left) vs HOBS-HSQC-TOCSY (right) spectra of region around δ 3.5-5.5 ppm acquired with a SW(^{13}C)=1 ppm and with 50% NUS. A-HOBS-HSQC-TOCSY was acquired with a mixing time of 60 ms (t_{exp}=14 min). All the other acquisition parameters as described in Figure 4.

Figure 6: 2D HOBS-HSQMBC spectrum of region around δ 3.5-5.5 ppm acquired with a SW(^{13}C)=5ppm, 32 scans per t_1 increment and optimized to $1/(2.^{n}J_{CH})$=8 Hz and with 50% NUS. All other parameters as describe in Figure 4. The total experimental time was about 2 hours.

Conclusions

- ✓ 1D and 2D HOBS methods allow the fast distinction and assignment of similar compounds exhibiting near-identical ^{1}H and ^{13}C NMR spectra.
- ✓ The combination of HOBS, spectral aliasing and NUS techniques in a single experiment afford ultra-high-resolved 2D NMR spectra with full sensitivity.
- ✓ Extremely small $\Delta\delta(^{1}H)$ and $\Delta\delta(^{13}C)$ values (1 and 5 ppb, respectively) can be simultaneously determined in short acquisition times.

References

[1] L. Castañar *et al.*, *Chem. Eur. J.*, **2015,** *21,* 7682.

[2] a) K. Zangger. *Prog. Nucl. Magn. Reson. Spectrosc,* **2014**, *15*, 49. b) R. W. Adams, *eMagRes,* **2014**, *3*, 295. c) L. Castañar, T. Parella, *Magn. Reson. Chem.*, **2015**, *53*, 399.

[3] a) K. Kazimierczuk, V. Y. Orekhov, *Angew. Chem. Int. Ed.*, **2011**, *50*, 5556. b) K. Kazimierczuk *et al.*, *J. Magn. Reson.*, **2006**, *179*, 323.

[4] a) G. B. B. Njock *et al.*, *Chim. Int. J. Chem*. **2010**, *64*, 235. b) A. Cotte *et al.*, *Chim. Int. J. Chem*. **2012**, *66*, 764. c) M. Pérez-Trujillo *et al.*, *Chem. Commun*. **2014**, *50*, 10214.

[5] a) L. Castañar, *et al.*, *Chem. Eur. J.* **2013**, *19*, 17283. b) J. Ying *et al.*, *J. Magn. Reson*. **2014**, *241*, 97. c) R. W. Adams *et al.*, *Chem. Commun*. **2014**, *50*, 2512.

[6] L. Castañar, *et al.*, *J. Magn. Reson*. **2014**, *238*, 63.

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