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 (Δδ) and to the wide J-coupling patterns. A successful characterization can be further complicated when trying to differentiate structural compounds exhibiting extremely small Δδ and similar J-coupling patterns between analogous protons, resulting in 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. This strategy is based on the combination of several resolution-enhanced techniques such as pure shift, non-uniform sampling (NUS) and spectral aliasing techniques (SA) into a single NMR experiment.

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 1H and 13C NMR spectra that were finally determined as three pairs of diastereoisomeric derivatives (Scheme 1).

### Results

#### High-resolved 1D NMR Spectra

At first glance, the analysis of conventional 1H 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 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 1H HOBS (Figure 1B,C) and the standard 1H HETCOR (Figure 3) spectra. Most of the 1H and 13C signals appear doubled and the fast distinction of extremely small Δδ confirm the presence of very similar compounds.

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

### Conclusions

- 1D and 2D HOBS methods allow the fast distinction and assignment of similar compounds exhibiting near-identical 1H and 13C 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 Δδ(1H) and Δδ(13C) values (1 ppm and 5 ppm, respectively) can be simultaneously determined in short acquisition time.

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