

# Band-selective pure shift $^1\text{H}$ NMR experiments with full sensitivity

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## Introduction

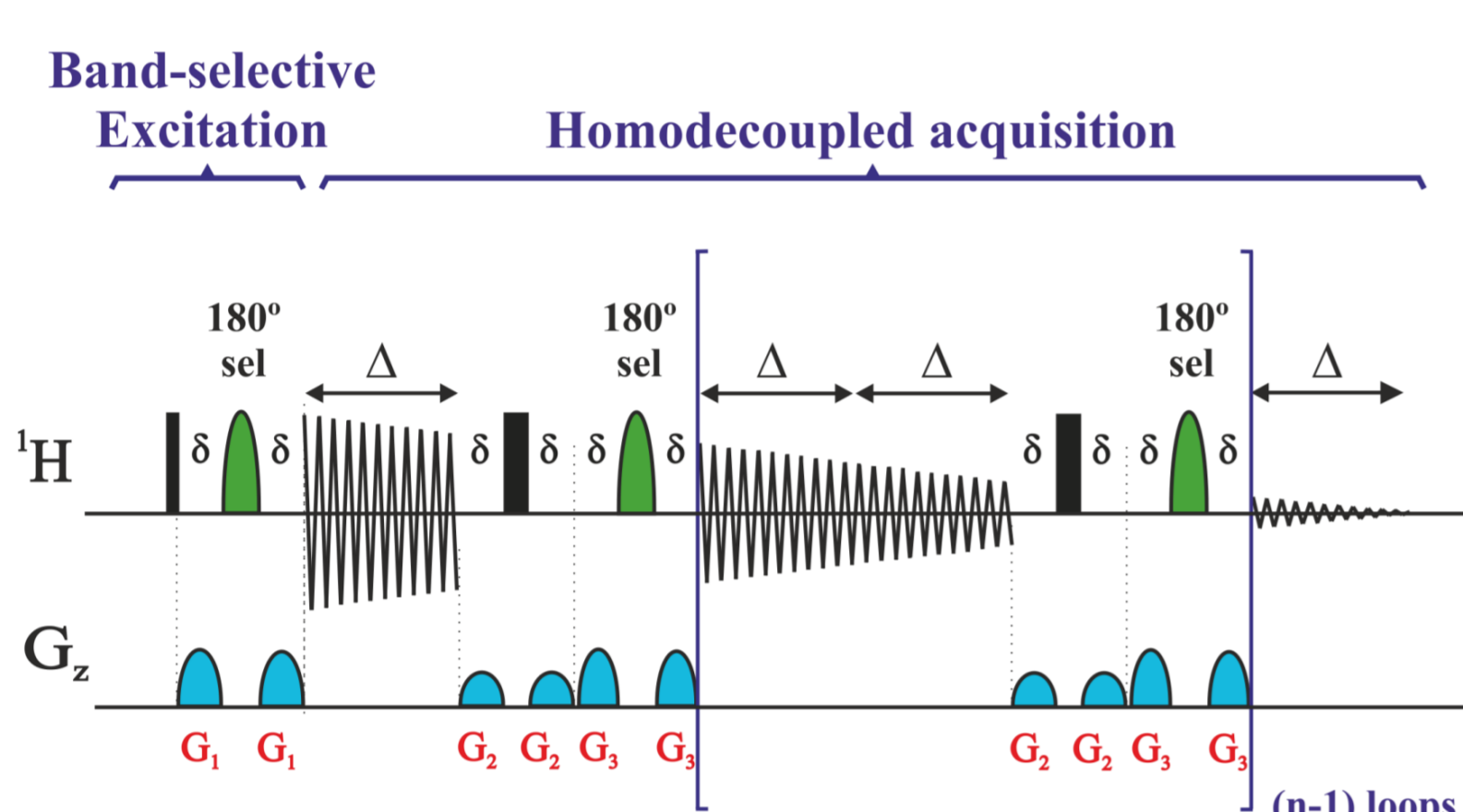
Over recent years, a significant interest has emerged to develop homodecoupled NMR techniques that offer enhanced resolution by simplifying the homonuclear  $J_{\text{HH}}$  pattern, and therefore reducing signal overlapping. Some years ago, the pseudo 2D Zangger-Sterk (ZS) method was proposed as a powerful tool to obtain high-resolved 1D  $^1\text{H}$  homodecoupled spectra<sup>1</sup> and it has been further refined and improved to obtain "pure shift" multidimensional NMR spectra.<sup>2,3</sup> The main challenge in this field relies in the design of new approaches which do not involve loss of sensitivity due to spatial selection.

Here we present a new NMR method based on the instant real-time 1D ZS experiment<sup>3</sup> for the fast acquisition of full-sensitive HOmodecoupled Band-Selective (HOBS)  $^1\text{H}$  NMR spectra<sup>4</sup>. Its implementation in 2D experiments and several practical applications to distinguish small chemical shift differences, such as found in enantiodifferentiation studies by using chiral solvating agents (CSAs)<sup>5</sup>, the analysis of individual signal intensity decays for measuring  $T_1/T_2$  relaxation times in overlapped signals<sup>6</sup> or the measurement of heteronuclear coupling constants from simplified multiplet patterns,<sup>7</sup> are described.

## HOBS Experiment

### Pulse Sequence

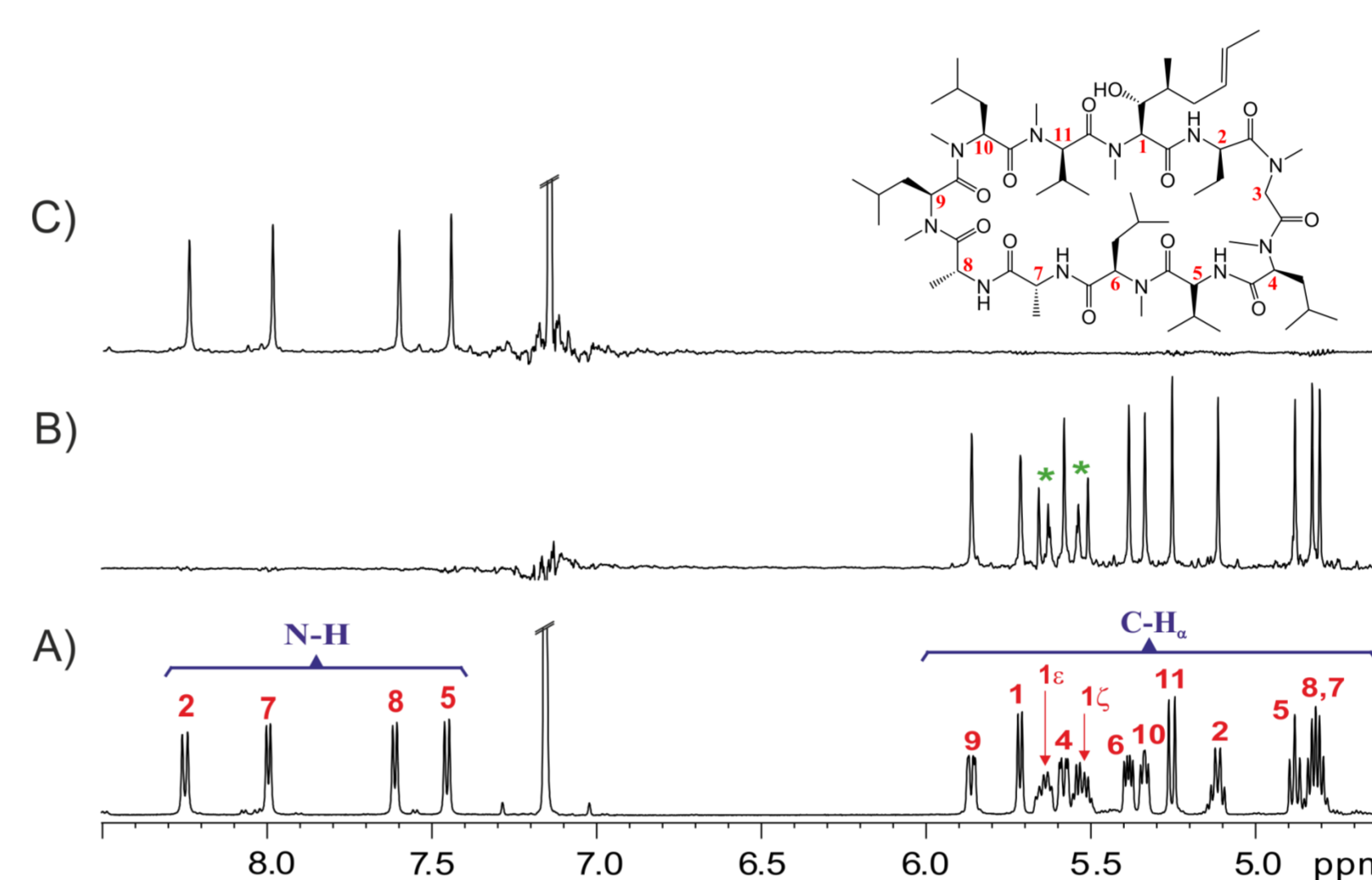
The spatial encoding gradients that are applied simultaneously with the selective  $180^\circ$  pulses in the original instant ZS scheme are omitted in the HOBS experiment, avoiding sensitivity losses.



**Figure 1:** Pulse scheme of the 1D HOBS experiment. Homodecoupling during acquisition is achieved by applying a pair of hard/semiselective  $180^\circ$   $^1\text{H}$  pulses at the middle of  $2\Delta=AQ/n$  periods, where  $AQ$  is the acquisition time and  $n$  the number of concatenated loops.  $\delta$  is the duration of a gradient and its recovery delay.  $G_x$ ,  $G_y$  and  $G_z$  gradients act as defocusing/refocusing coherence elements. The selectivity of the semiselective  $180^\circ$   $^1\text{H}$  pulse for both excitation and decoupling is set as a function of the selected region.

### NMR Spectra

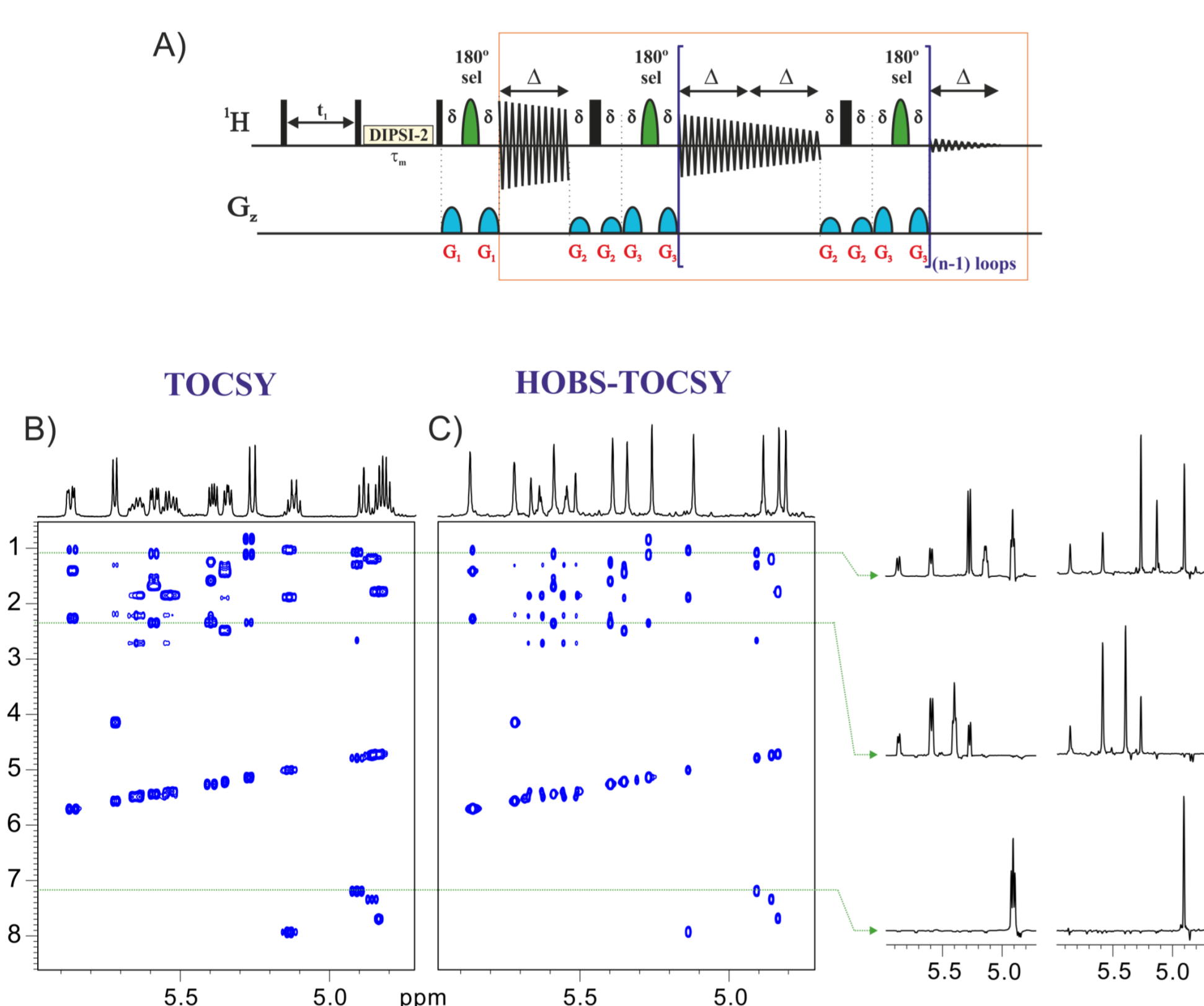
The HOBS method is a band/frequency-selective experiment which affords homodecoupled singlet signals in particular areas of the  $^1\text{H}$  spectrum whenever the protons are not mutually J-coupled.



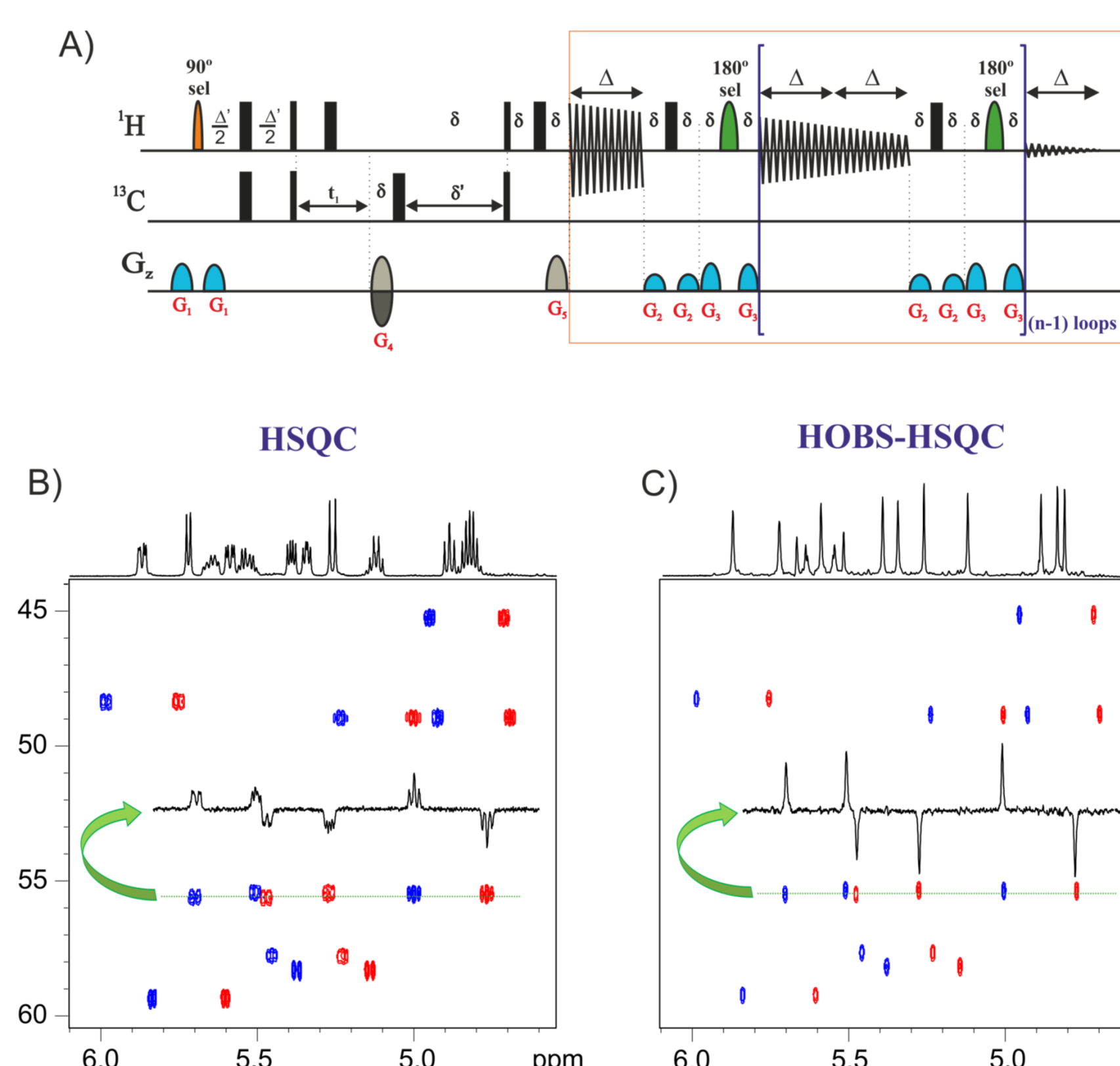
**Figure 2:** 600.13 MHz A) conventional and B,C) HOBS  $^1\text{H}$  NMR spectra of 25mM cyclosporine in  $\text{C}_6\text{D}_6$  (1) after selection of  $\text{H}_\alpha$  and NH regions, respectively. All spectra were recorded with the same receiver gain and with a single scan. HOBS spectra were acquired by applying 5ms  $180^\circ$  REBURP pulses for both excitation and decoupling. 8K data points were acquired using  $AQ=576\text{ms}$  and  $n=40$  ( $\Delta=7.2\text{ms}$ ). The strengths of  $G_x$ ,  $G_y$  and  $G_z$  were set to 12.3, 21.9 and 33.7 G/cm, respectively, with durations of 500 $\mu\text{s}$ . The asterisks marked in (B) stand for unavoidable non-decoupled effects of an AB spin system.

## Implementing HOBS in 2D NMR Experiments

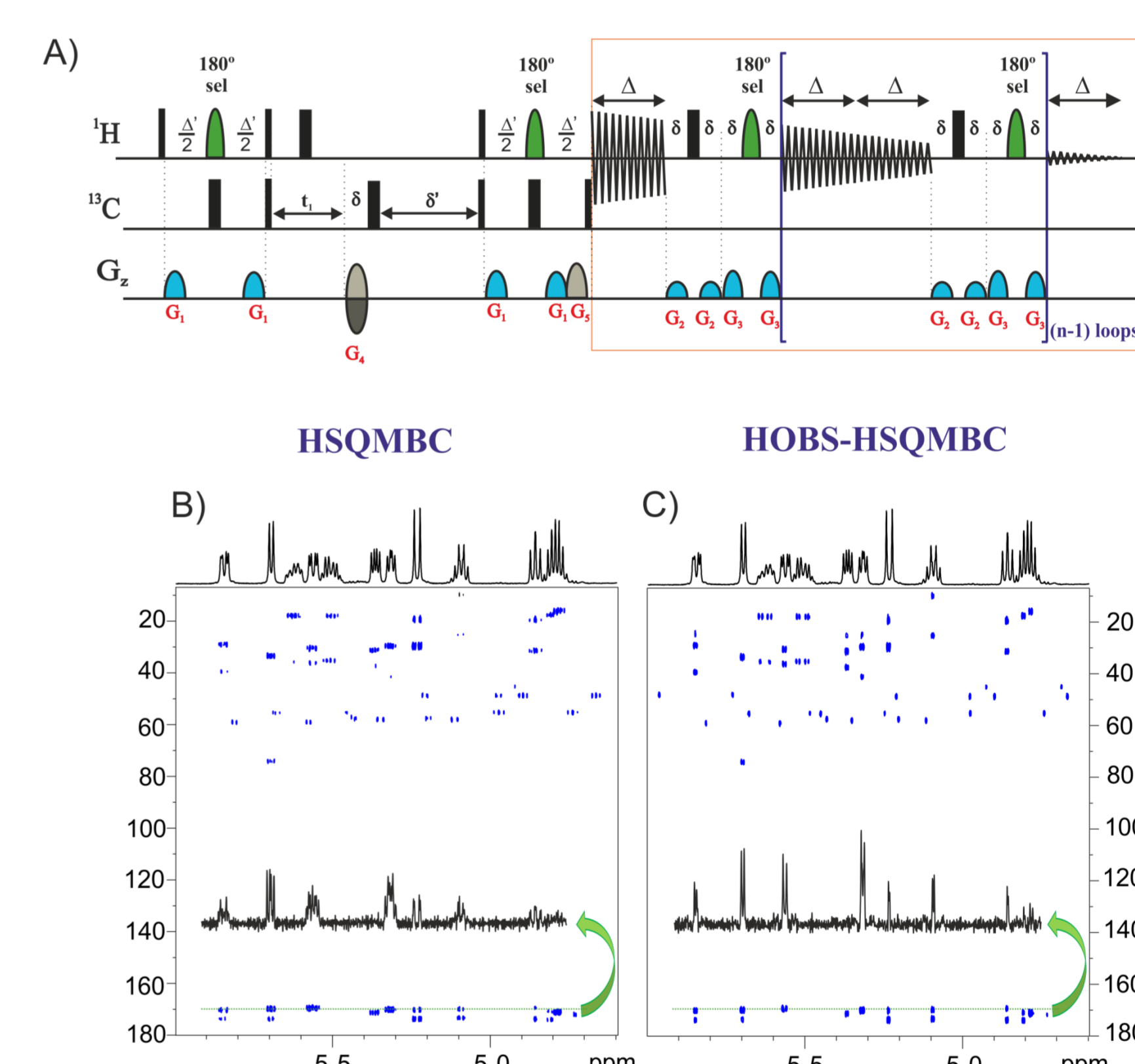
The implementation of the HOBS approach becomes reliable for a large number of multidimensional NMR experiments<sup>4,7</sup> and guarantees the rapid data acquisition of region-selective homodecoupled NMR spectra even for samples at low concentrations.



**Figure 4:** A) Pulse scheme of the HOBS-TOCSY experiment. B) TOCSY and C) HOBS-TOCSY spectra of 1 after selection of  $\text{H}_\alpha$  region. Four scans were collected for each 128  $t_1$  increments of 2K complex points and a mixing time ( $\tau_m$ ) of 60ms giving an experimental time of 13 min for each spectrum. Homodecoupling was achieved using an  $AQ=170\text{ms}$  and 20 loops ( $\Delta=4.3\text{ms}$ ) whereas all the other experimental parameters were as described in Figure 2.



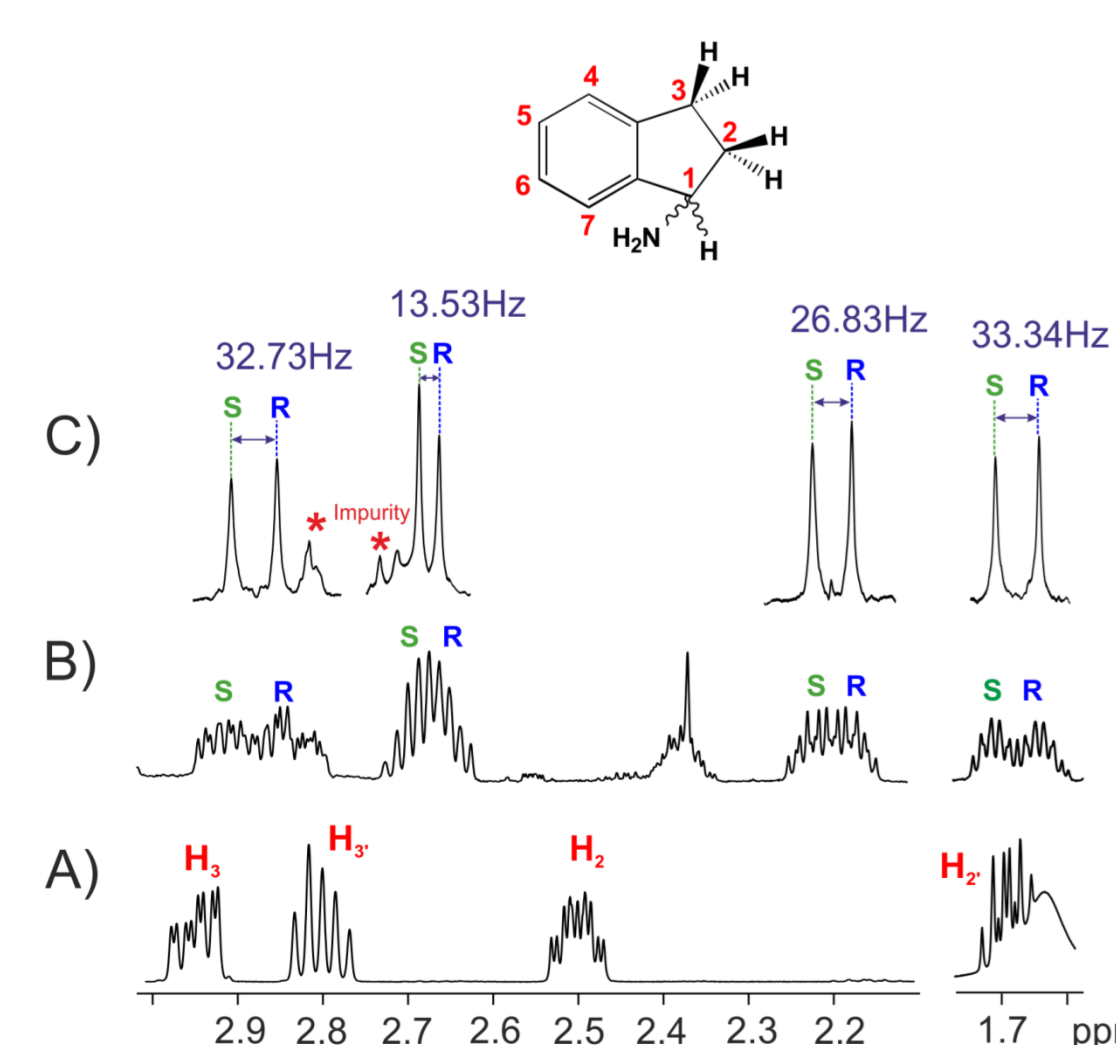
**Figure 5:** A) Pulse scheme of the F2-heterocoupled HOBS-HSQC experiment. B) conventional and C) HOBS-HSQC spectra of 1 optimized to 145Hz after selection of  $\text{H}_\alpha$  region. Two scans were collected for each one of the 64  $t_1$  increments of 2K complex data points. Homodecoupling was achieved using an  $AQ=570\text{ms}$  and  $n=50$  ( $\Delta=5.7\text{ms}$ ). The  $90^\circ$  and  $180^\circ$  band-selective pulses were EBURP-2 of 3.5ms and REBURP of 5.0ms, respectively. The experimental time for each 2D spectrum was of 5min.



**Figure 6:** A) Pulse scheme of the F2-heterocoupled HOBS-HSQMBC experiment. B) selHSQMBC and C) HOBS-HSQMBC spectra of 1 optimized to 8Hz after selection of  $\text{H}_\alpha$  region. Four scans were collected for each one of the 128  $t_1$  increments of 4K complex data points. The region-selective  $180^\circ$   $^1\text{H}$  pulse was a REBURP of 5ms. Homodecoupling was achieved using  $AQ=1.13\text{s}$  and  $n=20$  ( $\Delta=28.2\text{ms}$ ). The experimental time for each 2D spectrum was of 20min.

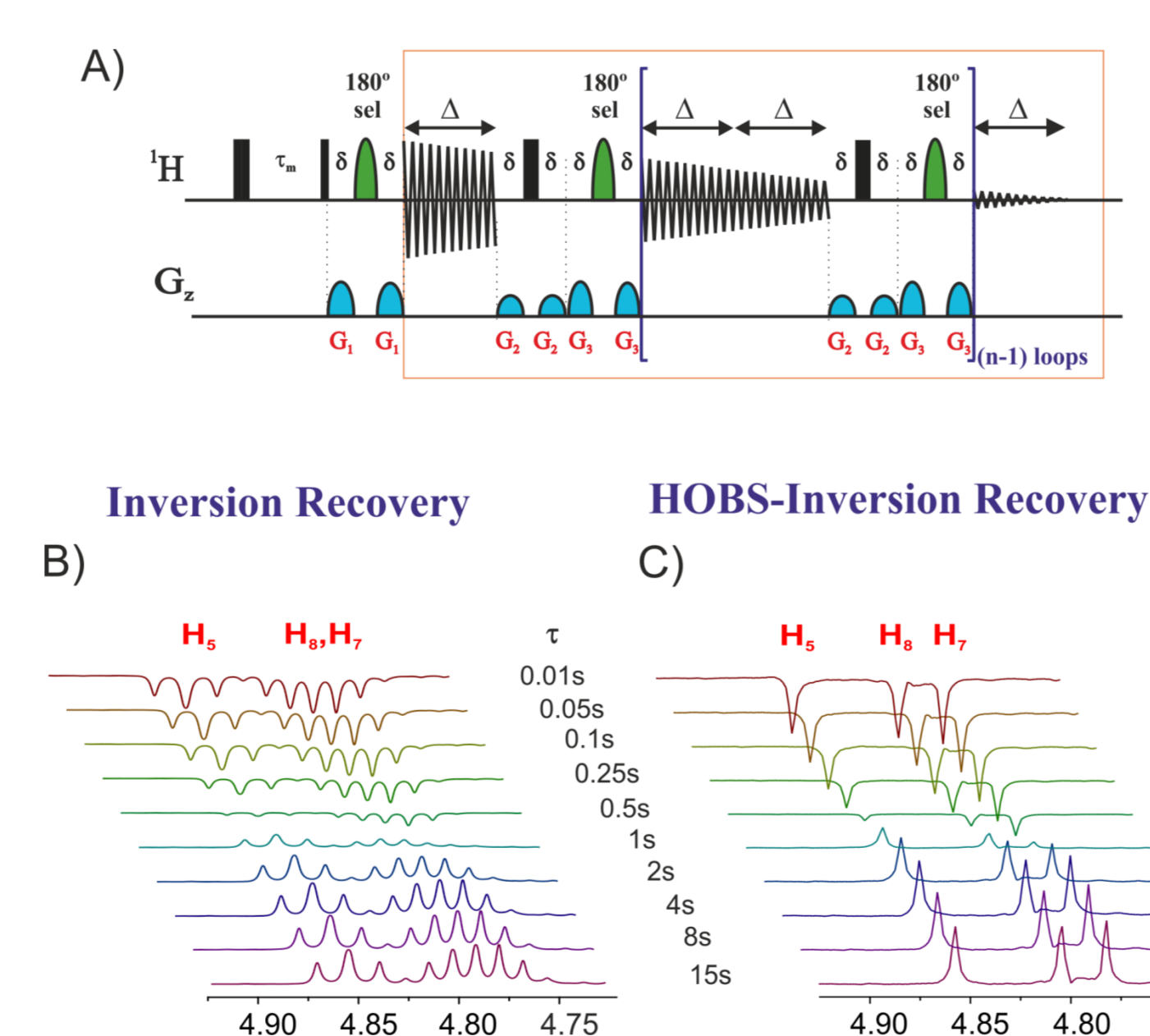
## HOBS Applications

### Fast and efficient enantiodifferentiation by using CSAs<sup>5</sup>



**Figure 7:** 600.13 MHz  $^1\text{H}$  NMR spectra of 50 mM (*R,S*)-1-aminoindan (1:1 proportion) in  $\text{CDCl}_3$ : 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 HOBS experiments (Gaussian-shaped  $180^\circ$  pulse of 20 ms,  $\Delta=18.93\text{ms}$ ,  $AQ=2.27\text{s}$ , and  $n=60$ ). All 1D spectra B and C were acquired with a single scan and plotted with the same vertical scale.

### Measurement of $T_1/T_2$ relaxation times in overlapped signals<sup>6</sup>



**Figure 8:** A) Pulse scheme of the 1D HOBS-IR experiment. Expansion of B) conventional IR and C) HOBS-IR spectra to determine  $T_1$  values for some overlapped  $\text{H}_\alpha$  protons of 1. Homodecoupling was achieved using a 5ms REBURP  $180^\circ$  pulse,  $\Delta=8.9\text{ms}$ ,  $AQ=569\text{ms}$  and  $n=32$ .

## Conclusions

The real-time HOBS method:

- ✓ yields homodecoupled NMR spectra of specific regions with an excellent spectral quality.
- ✓ guarantees fast acquisition and conventional data processing.
- ✓ affords spectra with full sensitivity and enhanced resolution.
- ✓ can be easily implemented into conventional mono- and multidimensional NMR experiments.

## References:

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(b) J. A. Aguilar et al. **2010**, *Angew. Chem. Intl. Ed.* 49, 3901-3903.
- <sup>3</sup> N. H. Meyer, K. Zangger **2013**, *Angew. Chem. Intl. Ed.* 52, 7143-7146.
- <sup>4</sup> L. Castañar et al. **2013**, *Chem. Eur. J.* 19, 17283-17286.
- <sup>5</sup> L. Castañar et al. **2014**, *ChemPhysChem.* 15, 854-857.
- <sup>6</sup> L. Castañar et al. **2014**, *J. Magn. Reson.* 244, 30-35.
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