GEMSTONE-ROESY: Ultra-selective, ultra-clean 1D rotating-frame Overhauser effect spectroscopy

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Introduction

2D NMR methods provide extensive structural and conformational information on molecules, but are often time-consuming. Their 1D ^{1}H selective analogues allow key information required for analysis to be extracted in a much shorter time. However, in ^{1}H NMR the narrow range of chemical shifts and the typically high signal multiplicity often cause multiplets to overlap, so that traditional selective excitation methods cannot select a single chemical site. A new 1D ultra-selective approach has recently been developed, named GEMSTONE[1] (Gradient-Enhanced Multiplet-Selective Targeted-Observation NMR Experiment), which enables the selection of a single signal even in the presence of severe multiplet overlap. Here, the novel GEMSTONE analogue of the 1D ROESY experiment is introduced, providing unambiguous through-space correlations.

GEMSTONE mechanism

The ultra-selective GEMSTONE experiment (Figure 1) provides selectivity akin to the CSSF (Chemical Shift Selective Filter)[2] experiment, but as it does so in a single scan it retains the full time advantage of selective 1D over 2D experiments.

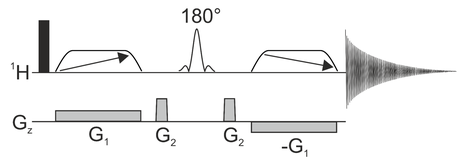
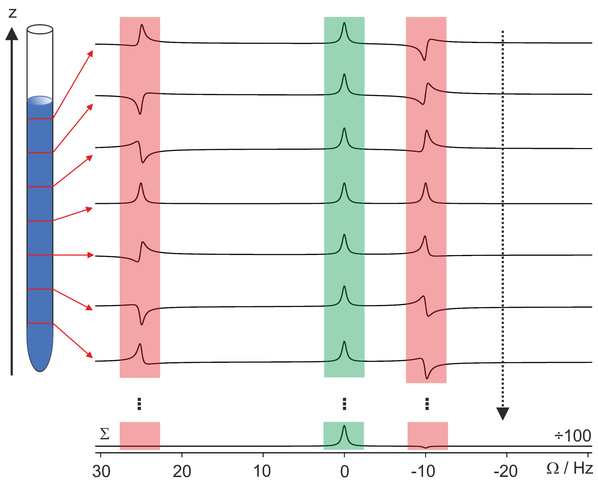


Figure 1: GEMSTONE pulse sequence. The narrow rectangle represents a hard 90° radiofrequency pulse. The open trapezoids with directional arrows are adiabatic 180° pulses used for GEMSTONE selection. Pulsed field gradients are represented by G_z. The labelled 180° pulse shape denotes a band-selective refocusing rSNOB pulse.



The swept-frequency pulses spatially encode all signals in the NMR tube, as demonstrated schematically in Figure 2:

- **on-resonance** signals retain the same phase throughout the NMR tube,
- **off-resonance** signals acquire a spatially-dependent phase.

GEMSTONE averages **off-resonance** signal to zero over the length of the NMR tube leaving just the in-phase **on-resonance** signal.

Figure 2: Simulated spectra of slices through the sample during a GEMSTONE experiment. 100 slices were summed to provide the spectrum shown at the bottom. The on-resonance signals are highlighted in green and off-resonance signals are highlighted in red.

GEMSTONE-ROESY pulse sequence

GEMSTONE-ROESY (Figure 3) enables selection of a single signal and provides through-space interactions where the NOE provides little or no intelligible data. GEMSTONE-ROESY allows the assignment of previously ambiguous through-space interactions, enabling molecular conformation and configuration to be established. Ultra-clean spectra free of suppression artefacts, due to the additional gradient encoding, are acquired allowing ROE signals to be easily identified.

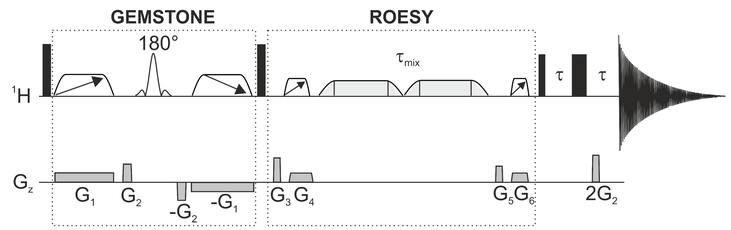
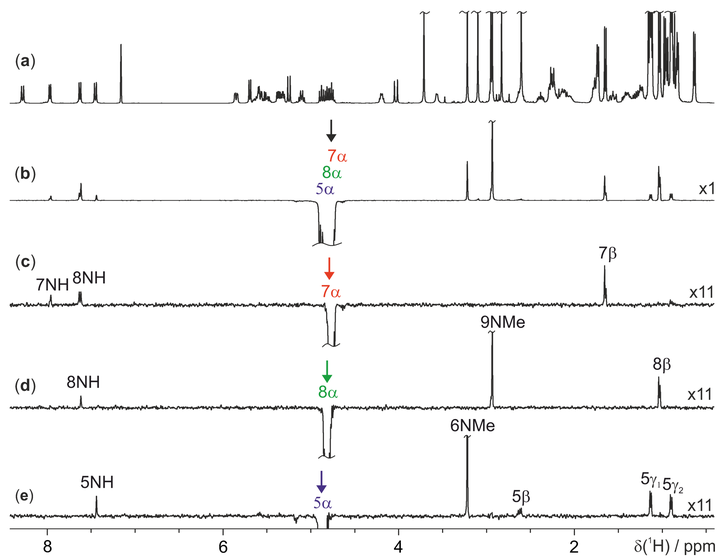


Figure 3: GEMSTONE-ROESY pulse sequence. The EASY-ROESY[3] spin-lock is shown by light grey trapezoids. ZQC suppression elements are represented by open trapezoids with directional arrows. The delay τ is sufficient for the gradient pulse and recovery delay.

GEMSTONE-ROESY: cyclosporin



The newly developed GEMSTONE-ROESY experiment provides unambiguous through-space correlations allowing 3D molecular conformation to be determined. Figure 4 shows the utility of this ultra-selective ROESY technique where 4 overlapped alpha proton signals in cyclosporin have been individually targeted. ROE signals of each amino acid residue can be assigned and it provides insight into the adjacent amino acid residues.

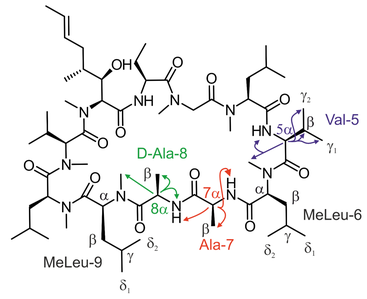


Figure 4: (a) Conventional ^{1}H NMR spectrum of cyclosporin in C_6D_6. (b) Conventional 1D selective EASY-ROESY exciting at 4.82 ppm, and (c-e) GEMSTONE-ROESY spectra exciting at 4.77 (Ala-7α), 4.82 (D-Ala-8α), and 4.88 (Val-5α) ppm, respectively. Spectra (c-e) are scaled by a factor of 11 to match the intensity of (b); spectrum (a) is not to scale.

GEMSTONE-ROESY: Lacto-*N*-difucohexaose I

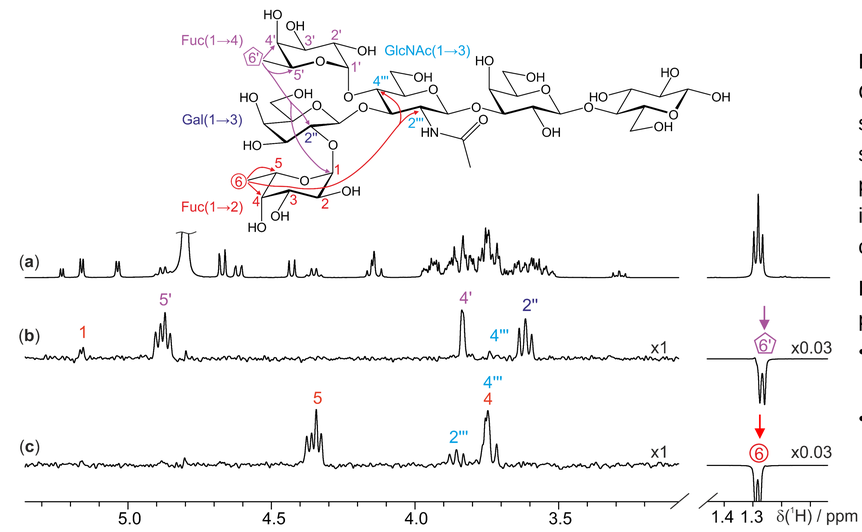


Figure 5 demonstrates the use of GEMSTONE-ROESY in the structural study of lacto-*N*-difucohexaose I, a structurally complex oligosaccharide present in breast milk. 3D studies provide insight into the sugar configurations and conformations.

Here, the GEMSTONE-ROESY spectra provide:

- clear ROE correlations within each monosaccharide unit, and
- evidence of through-space contacts with neighbouring monosaccharide units.

Figure 5: (a) Conventional ^{1}H NMR spectrum of lacto-*N*-difucohexaose I in D_2O. (b and c) GEMSTONE-ROESY spectra selecting H6' and H6, respectively; the separation between the signals selected is only 6 Hz. The selected signal (right hand side) is scaled by a factor of 0.03.

References

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Acknowledgments

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