Electronic Supplementary Material (ESI) for ChemComm.

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

Electronic Supporting Information

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Contents

A. Experimental details

1. Pulse sequence

Further details of the new GEMSTONE-ROESY method introduced in the main paper are provided here. The schematic pulse sequence is shown in Figure S1, and the Bruker pulse program code is provided in Section D.

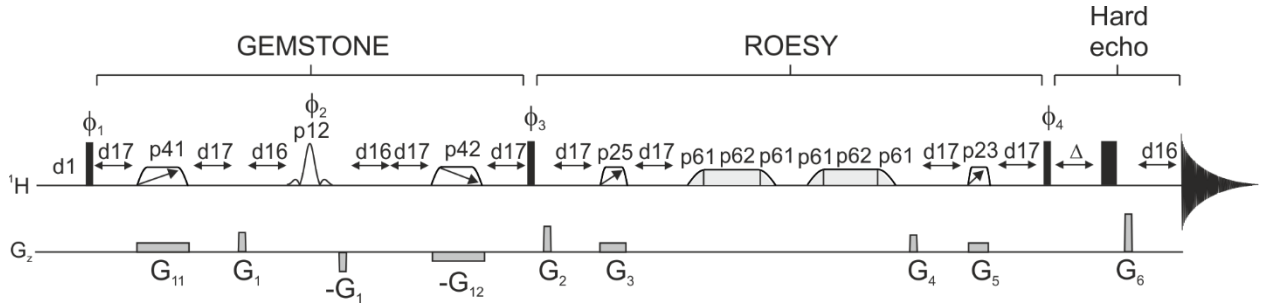

Figure S1: Schematic representation of the GEMSTONE-ROESY pulse sequence for Bruker implementation. The sequence incorporates elements from the recently published GEMSTONE[1] and the established 'EASY-ROESY'[2] methods.

In Figure S1, narrow and wide rectangles represent hard 90° and 180° radiofrequency (RF) pulses, respectively. The white shaped pulse represents a moderately selective 180° refocusing pulse with a duration p12; typically, an Rsnob pulse is used. Open trapezoids with directional arrows represent low power 180° swept-frequency pulses; these are used in both GEMSTONE (p41 and p42), for spatiotemporal encoding, and in ROESY, for zero-quantum coherence (ZQC) suppression (p23 and p25). The EASY-ROESY spin-lock is implemented by applying two ramped continuous wave RF irradiations with durations p61 (adiabatic ramps) and p62 (constant amplitude). The total spin-lock mixing time is divided into two periods of off-resonance RF irradiation, applied at high and low frequency offsets, respectively, in order to minimise offset effects on the intensities of the ROEs.[2] TOCSY interference is attenuated by setting the offsets of the spin-locks outside the spectral window, with careful selection of the spin-lock angle; typically, an angle between 45° and 60° is used.

Field gradients G_{11} and G_{12} are applied during the swept-frequency pulses in GEMSTONE (p41 and p42) to provide the spatiotemporal averaging responsible for the selectivity of the experiment. Gradients G_2 and G_4 are purge gradients which dephase the remaining transverse magnetisation. Gradients G_1 and G_6, where G_6 is set to $2G_1$, enforce the coherence transfer pathway (CTP), which allows retention of the wanted signals while dephasing unwanted signals, leaving ultra-clean spectra. Gradient stabilisation delays are defined by d16 and d17. The time Δ in the hard spin echo is determined by the duration of the G_6 gradient (p16) and the gradient stabilization delay (d16). Phase and intensity anomalies are occasionally present in the observed ROE signals (see section B5).

Table S1: Phase cycling of GEMSTONE-ROESY.

ϕ_1	x_{16}, $-x_{16}$
ϕ_2	x_4, y_4, $-x_4$ $-y_4$
ϕ_3	x_2, $-x_2$,
ϕ_4	x, -x,
$\phi_{receiver}$	$(x, -x, -x, x, -x, x, x, -x)_2$ $(-x, x, x, -x, x, -x, -x, x)_2$

2. Guide to experimental setup

A recommended protocol to acquiring GEMSTONE-ROESY spectra is provided here.

1) Acquire a ^{1}H NMR spectrum and calibrate the 90° hard pulse.
2) Acquire a pure shift NMR spectrum to give exact chemical shift information for each signal to be targeted by GEMSTONE. There are several pure shift methods available [3–6], all of which will provide the chemical shift information required for GEMSTONE. Here, the PSYCHE interferogram pure shift method was used producing a broadband pure shift spectrum. Alternatively, using a band-selective method for the region of interest would provide the required information with maximum sensitivity. In some cases, using Zangger-Sterk or BIRD active spin refocusing elements may be appropriate. As spectral purity is usually not of high importance when determining chemical shifts with pure shift methods, real-time or semi-real time acquisition with a small number of data chunks can be used to minimise data acquisition time.
3) Once the chemical shift of the signal to be targeted is known, the GEMSTONE parameters can be set. The user should optimise and run the GEMSTONE experiment prior to running the GEMSTONE-ROESY sequence, to confirm that only one signal is excited. In GEMSTONE, there are four main parameters to optimise. These are the bandwidth of the swept-frequency pulses (CNST51), the duration of the swept-frequency pulses (CNST52), the strength of the respective gradients (GPZ11 and GPZ12) applied in conjunction with the swept-frequency pulses, and the bandwidth of the band-selective pulse (CNST50). The pulse sequences provided are compatible with the Bruker Topspin Wavemaker tool, which enables the automatic calculation of pulse durations and power levels based on the calibrated 90° hard pulse power and duration, and selected variables. For GEMSTONE-ROESY, GEMSTONE and ZQC suppression elements are Wavemaker compatible.
    - The selectivity of the GEMSTONE experiment is controlled by the total duration of the swept-frequency pulses, which should be tuned by the user to achieve the best results. A longer duration results in increased selectivity at the expense of sensitivity. The duration suggested for each swept-frequency pulse is between 50 and 150 ms.
    - The user should set the bandwidth of the swept-frequency pulses to encompass a spectral width approximately 20% greater than the spectral region covered by the signals, as this will ensure that all signals experience the effects of the spatio-temporal encoding. The field gradient profile should be matched to cover the bandwidth of the swept-frequency pulses. The field gradient strength will need to be calibrated for a given probe's RF coil length to give clean signal selection.
    - The semi-selective 180° pulse acts only to refocus the effects of J-modulation; the pulse does not control the selectivity of the experiment. Therefore, the bandwidth of the selective pulse should be as wide as possible without inverting pairs of coupled spins, to prevent excessive relaxation. The user can define the shape of the pulse using Wavemaker.
4) The setup of EASY-ROESY is described in prior literature.[2,7,8] The user can control the RF field strength and the angle of the spin-lock; calculations encoded into the sequence will use this information to calculate the frequency offset to apply the spin-lock. Manipulating these parameters can limit TOCSY interference. If TOCSY interference is strong, a more acute spin-lock angle should be used in addition to reducing the RF field strength of the spin-lock.[8] ZQC suppression elements were set here to 50 and 30 ms, using a 60 kHz bandwidth with a matched simultaneous gradient, as incorporated by Boros *et al.* and advised by Thrippleton and Keeler.[7,9]

3. Experiment parameters

All experimental data for this paper are freely available for download from https://doi.org/10.48420/21905049.

All spectra were recorded on Bruker spectrometers and processed using Bruker Topspin software package (version 3.6.2 or 4.1.4).

Cyclosporin sample

The sample was prepared by dissolving 84 mg of commercial cyclosporin (Bio Basic) in 1 mL of C_6D_6 to give approximately 70 mM final concentration. All spectra were recorded at 293 K on a Bruker Avance III HD 400 MHz spectrometer with a 5 mm BBFO probe equipped with a *z*-gradient coil with a maximum nominal gradient strength of 53 G cm^{-1}. For all experiments, the duration of the ^{1}H hard 90° pulse was 13.3 µs.

The conventional ^{1}H NMR spectrum was acquired with a spectral width of 8000 Hz (20 ppm), the carrier frequency was set to 1800 Hz (4.5 ppm), and a time domain data length (TD) of 16384 points was used. The experiment was acquired with 8 scans and a recovery delay (d1) of 3 s. Prior to Fourier transformation, zero-filling to 65536 complex points was applied, along with Gaussian weighting using a LB of –0.01 Hz and GB of 0.008.

GEMSTONE-ROESY spectra were acquired with a spectral width of 8000 Hz (20 ppm) and a time domain data length (TD) of 16384 points. The carrier frequency was set to the exact chemical shifts of the selected signals (exact parameters used can be found in Table S2). The experiments were acquired with 256 scans and a recovery delay (d1) of 3 s. Prior to Fourier transformation, zero-filling to 65536 complex points was applied, along with Gaussian weighting using a LB of –0.01 Hz and GB of 0.0008. For the GEMSTONE element, WURST-80 adiabatic swept-frequency 180° pulses (Q-factor of 11 and step size of 5) were used, with 10 kHz bandwidth and duration (p41 and p42) approximately 100 ms (see Table S2). An Rsnob selective 180° refocussing pulse was used, with a bandwidth of 200 Hz. The spatial encoding gradients of opposite signs (G_{11} and G_{12}) applied in conjunction with the respective adiabatic pulses were set to 1.08 G cm^{-1} to match the effective bandwidth of the adiabatic pulses. A standard ROESY spin-lock mixing time of 200 ms was used, 100 ms at each frequency offset. The spin-lock used an RF field strength of 6400 Hz and were locked at an angle (θ) by applying the magnetisation at an offset depending on the equation $\Delta = \pm(\gamma B_1)/\tan(\theta)$; for these experiments θ was set to 50°. Pulsed field gradients G_1, $-G_1$ and G_6 were used to select the desired CTP, with amplitudes of 3.9, –3.9 and 7.9 G cm^{-1}, respectively, a duration (p16) of 0.2 ms, and a smoothed rectangular shape (SMSQ10.100). G_2 and G_4 were used as homospoil gradients and set to 24.9 and 16.4 G cm^{-1}, respectively. The ZQC suppression elements (p25 and p23) were set at 50 and 30 ms with a bandwidth of 60 kHz. Simultaneous gradients (G_3 and G_5) of 5.7 and 5.2 G cm^{-1} were applied. The pulsed field gradient recovery delays (d16 and d17) were set to 0.2 and 1 ms, respectively. The gradient duration p16 and recovery delay d16 were set relatively short to reduce the total hard echo duration (Δ).

The conventional 1D selective EASY-ROESY experiment (Figure 1b) was acquired using a band-selective Rsnob pulse of 20 Hz. All other acquisition and processing parameters were kept consistent with those used in the GEMSTONE-ROESY experiments.

Table S2: GEMSTONE parameters used for cyclosporin spectra. A swept-frequency pulse (p41 and p42) bandwidth of 10 kHz and simultaneous gradient (G_{11} and $-G_{12}$) of 1.08 G cm^{-1} was used in all experiments.

Figure	Chemical shift of selected signal / ppm	Frequency of selected signal / Hz	Swept-frequency pulse duration / ms	Band-selective pulse bandwidth / Hz
1c, S3c	4.78	1911.9	100	200
1d, S3e	4.82	1927.7	100	200
1e, S3g, S4c	4.88	1952.9	100	200
S3b	4.78	1911.9	100	200
S3d	4.82	1927.7	100	200
S3f	4.88	1952.9	100	200
S4b	4.82	1927.7	100	200
S5b	2.18	872.7	110	200
S5c	2.25	900.8	100	200
S5d	5.34	2134.5	100	200
S5e	5.39	2154.0	100	200

LNDFH I sample

A sample of LNDFH I (BioCarb Chemicals, Lund, Sweden) was prepared by dissolving 10.2 mg in 0.4 mL D_2O to give approximately 25 mM final concentration; trimethylsilylpropanoic acid sodium salt was added as a reference. All spectra were recorded at 295 K on a Bruker Avance III HD 400 MHz spectrometer with a 5 mm N_2 Prodigy cryoprobe equipped with a *z*-gradient coil with a maximum nominal gradient strength of 53 G cm^{-1}. For all experiments, the duration of the ^{1}H hard 90° pulse was 12.35 µs.

The conventional ^{1}H NMR spectrum was acquired with a spectral width of 5000 Hz (12.5 ppm), the carrier frequency was set to 1200 Hz (3.0 ppm), and a time domain data length (TD) of 32768 points was used. The experiment was acquired with 1 scan and a recovery delay (d1) of 3 s. Prior to Fourier transformation, zero-filling to 65536 complex points was applied, along with Gaussian weighting using a LB of –0.01 Hz and GB of 0.013.

GEMSTONE-ROESY spectra were acquired with a spectral width of 8000 Hz (20.0 ppm) and a time domain data length (TD) of 16384 points. The carrier frequency was set to the exact chemical shifts of the selected signals (exact parameters used can be found in Table S2). The experiments were acquired with 512 scans and a recovery delay (d1) of 3 s. Prior to Fourier transformation, zero-filling to 65536 complex points was applied, along with Gaussian weighting using a LB of –0.01 Hz and GB of 0.006. For the GEMSTONE element, WURST-80 adiabatic swept-frequency 180° pulses (Q-factor of 11 and step size of 5) were used, with a 5 kHz bandwidth and duration (p41 and p42) of approximately 120 ms (see Table S2). An Rsnob selective 180° refocussing pulse was used, with a bandwidth of 200 Hz. The spatial encoding gradients of opposite signs (G_{11} and G_{12}) applied in conjunction with the respective adiabatic pulses were set to 0.7 G cm^{-1} to match the effective bandwidth of the adiabatic pulses. A standard ROESY spin-lock mixing time of 200 ms was used, 100 ms at each frequency offset. The spin-lock used an RF field strength of 6400 Hz and the magnetisation was locked at an angle of 50° to the *z*-axis. Pulsed field gradients G_1, $-G_1$ and G_6 were used to select the desired CTP, with amplitudes of 3.9, –3.9 and 7.9 G cm^{-1}, respectively, a duration (p16) of 0.2 ms, and a smoothed rectangular shape (SMSQ10.100). Gradients G_2 and G_4 were used as homospoil gradients and set to 24.9 and 16.4 G cm^{-1}, respectively. The ZQC suppression elements (p25 and p23) were set at 50 and 30 ms with a bandwidth of 60 kHz. Simultaneous gradients (G_3 and G_5) of 5.7 and 5.2 G cm^{-1} were applied. The

pulsed field gradient recovery delays (d16 and d17) were set to 0.2 and 1 ms, respectively. The gradient duration p16 and recovery delay d16 were set relatively short to reduce the total hard echo duration (Δ).

The conventional 1D selective EASY-ROESY experiment (Figure 3c) was acquired using a band-selective Rsnob pulse of 10 Hz. All other acquisition and processing parameters were kept consistent with those used in the GEMSTONE-ROESY experiments.

Table S3: GEMSTONE parameters used for LNDFH I spectra acquired. A swept-frequency pulse (p41 and p42) bandwidth of 5 kHz and simultaneous gradient (G_{11} and $-G_{12}$) of 0.7 G cm^{-1} was used in all experiments.

Figure	Chemical shift of selected signal/ ppm	Frequency of selected signal/ Hz	Swept-frequency pulse duration/ ms	Band-selective pulse bandwidth/ Hz
3c (+ **S8c**)	1.27	506.9	130	200
3d (+ **S8e**)	1.28	513.0	120	200
S8b	1.27	506.9	130	200
S8d	1.28	513.0	120	200

B. Cyclosporin

1. ^{1}H and pure shift NMR spectra

Figure S2 shows the ^{1}H and PSYCHE[10] pure shift NMR spectra of cyclosporin used to determine the exact chemical shifts of the signals to be targeted in the GEMSTONE experiments.

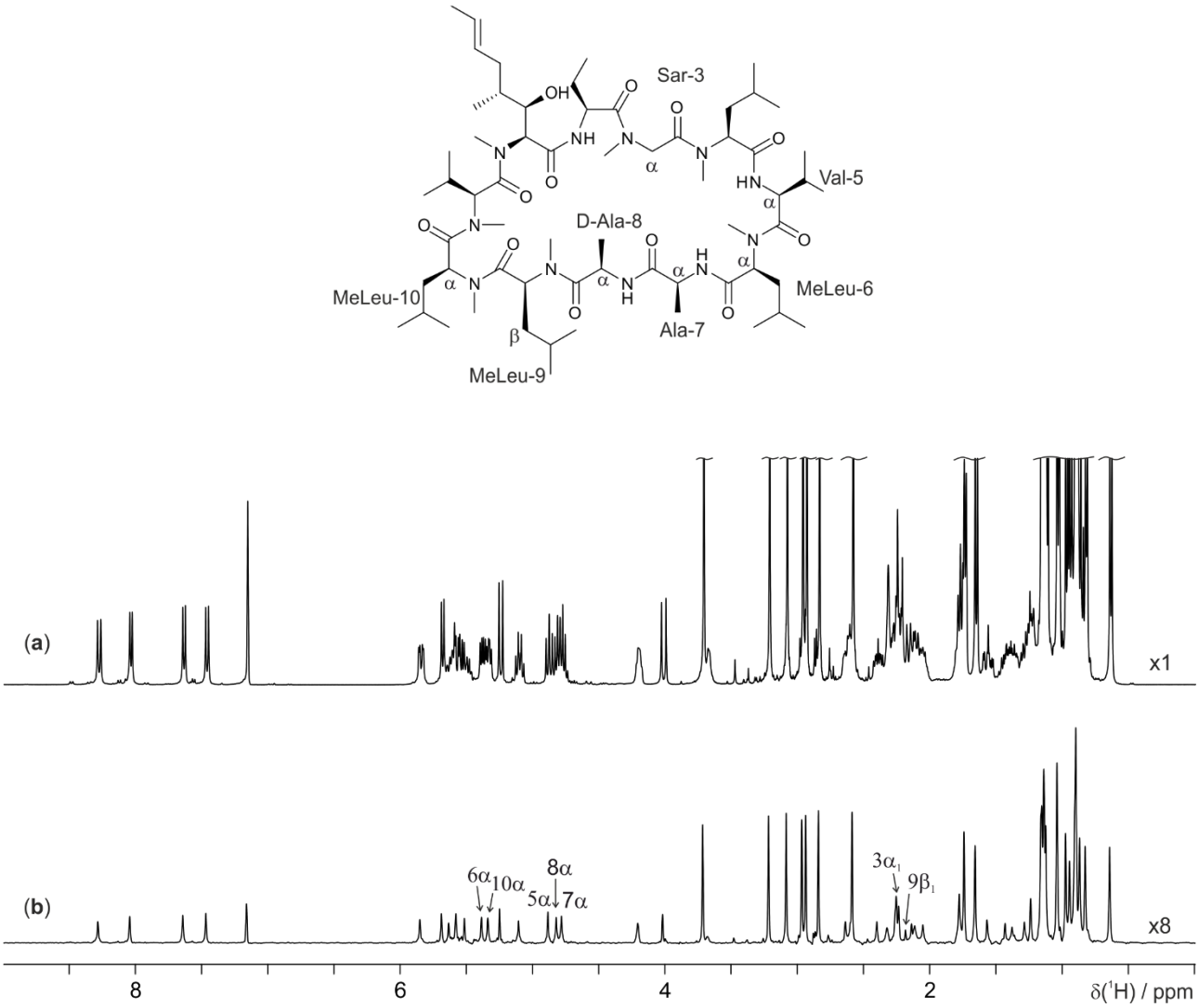

Figure S2: 400 MHz ^{1}H NMR spectra of 70 mM cyclosporin in C_6D_6. Molecular structure with relevant assignments[11] is shown above the spectra. (**a**) Conventional ^{1}H 1D spectrum and (**b**) PSYCHE pure shift spectra acquired using a spectral width of 5000 Hz (12.5 ppm), a chunk duration of 20 ms, and 20 chunks. A 30 ms double saltire pulse with a bandwidth of 10 kHz and a 20° flip angle was used with a simultaneous gradient of 0.53 G cm^{-1}. An AU processing macro, 'pshift' (freely available from https://www.nmr.chemistry.manchester.ac.uk/?q=node/372), was used to convert the pseudo-2D pure shift data into a 1D FID. Prior to Fourier transformation, zero-filling to 65536 complex points was applied, along with Gaussian weighting using a LB of –0.01 Hz and GB of 0. 008.

2. NOE *vs* ROE

GEMSTONE-ROESY offers an alternative ultra-selective method to observe through-space interactions where the NOEs are negligible. This is demonstrated in Figure S3, where GEMSTONE-NOESY (Figures S3b, 3d and 3f) fails to provide most through-space interactions from the selected α-protons.

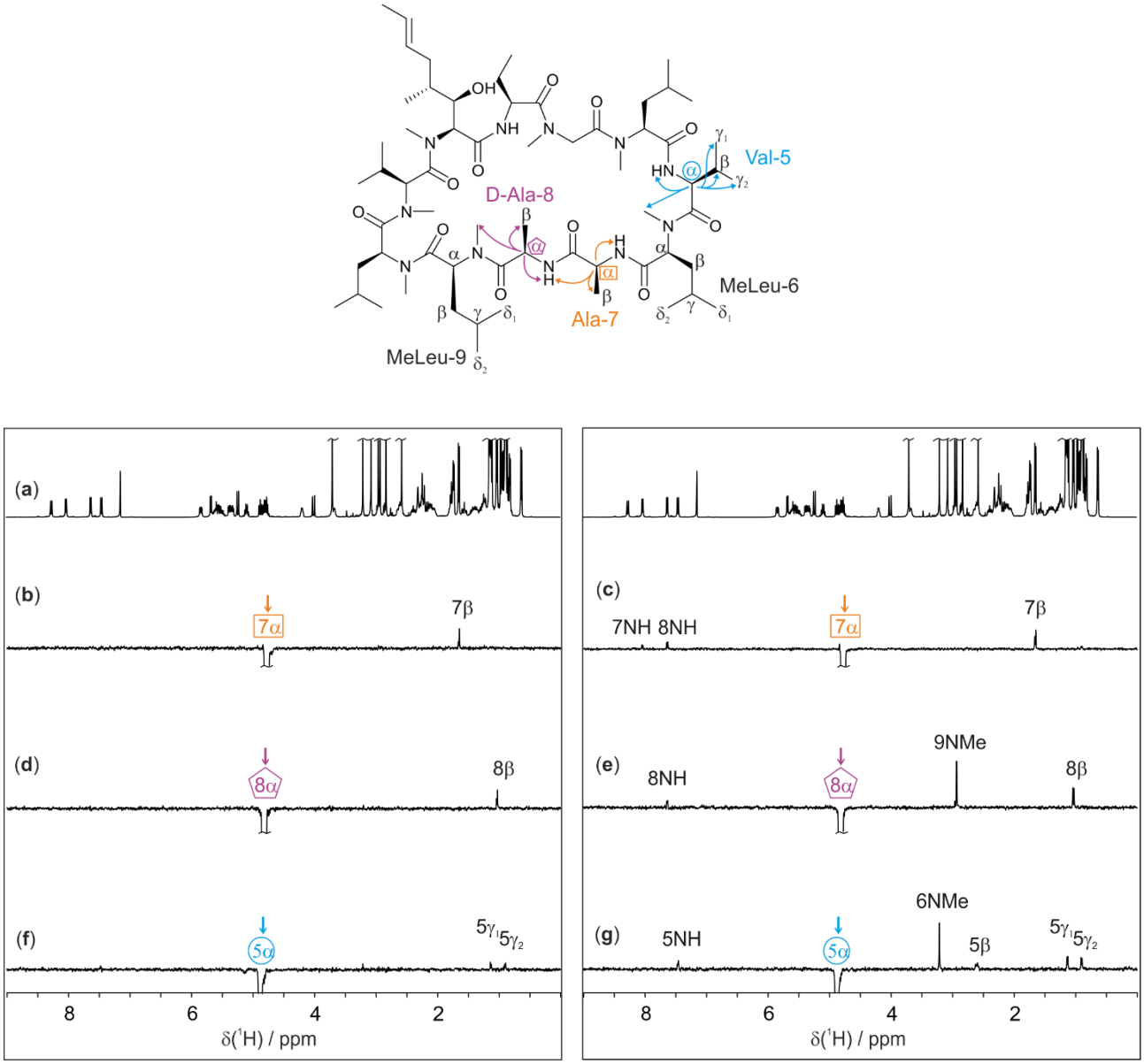

Figure S3: 400 MHz ^{1}H NMR spectra of 70 mM cyclosporin in C_6D_6. Molecular structure with relevant assignments[11] is shown above the spectra with arrows representing the ^{1}H-^{1}H ROEs observed; for simplicity, individual protons are not shown. (**a**) Conventional ^{1}H 1D spectrum. (**b, d, f**) GEMSTONE-NOESY spectra selecting for Ala-7α, D-Ala-8α, and Val-5α, respectively. The total NOESY mixing time was 400 ms. All other processing and acquisition parameters were kept consistent with the GEMSTONE-ROESY experiments. (**c, e, g**) GEMSTONE-ROESY spectra selecting for Ala-7α, D-Ala-8α, and Val-5α, respectively. All GEMSTONE parameters are provided in Table S2.

3. Gradient CTP selection

Figure S4 demonstrates how the incorporation of the CTP gradients (G_1 and G_6 in Figure S1) to select the desired CTP greatly improves the quality of GEMSTONE-ROESY spectra.[8,12] If coherence selection is achieved solely via phase cycling, then large subtraction artefacts are observed (Figure S4b), hindering the identification of genuine ROEs. If both phase cycling and pulsed field gradients are used to reinforce the CTP, subtraction artefacts are removed, providing an ultra-clean ROESY spectrum (Figure S4b) enabling clear, unambiguous assignment of ROE signals.

A sensitivity penalty is paid when PFGs are used as only a single CTP is reinforced. Approximately half the intensity is observed in comparison to an experiment which does not use PFGs for CTP reinforcement; small additional losses are seen due to convection and diffusion.

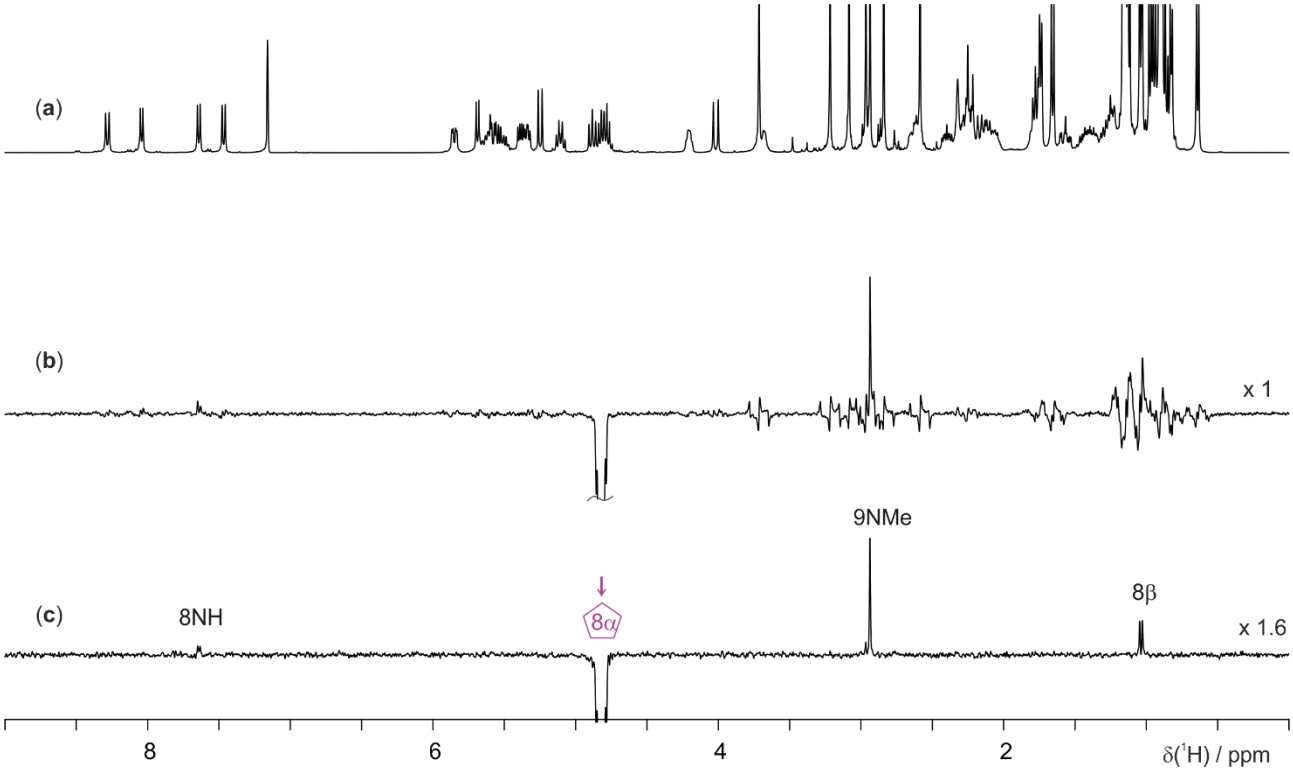

Figure S4: 400 MHz ^{1}H NMR spectra of 70 mM cyclosporin in C_6D_6. (**a**) Conventional ^{1}H 1D spectrum. (**b**) 1D GEMSTONE-ROESY spectrum without gradient-selection for CTP reinforcement. Here, the hard echo was omitted and gradients G_1 were set at 1:1 ratio around the semi-selective 180° pulse. All other parameters remained as stated in Section A and no further changes to the pulse sequence were made. (**c**) 1D GEMSTONE-ROESY spectrum with gradients to reinforce the CTP. The pulse sequence and parameters used were exactly as stated in Section A.

4. Further GEMSTONE-ROESY data

Further examples of GEMSTONE-ROESY data for cyclosporin are provided in Figure S5. The signals selected here are difficult to select cleanly using traditional 1D selective methods.

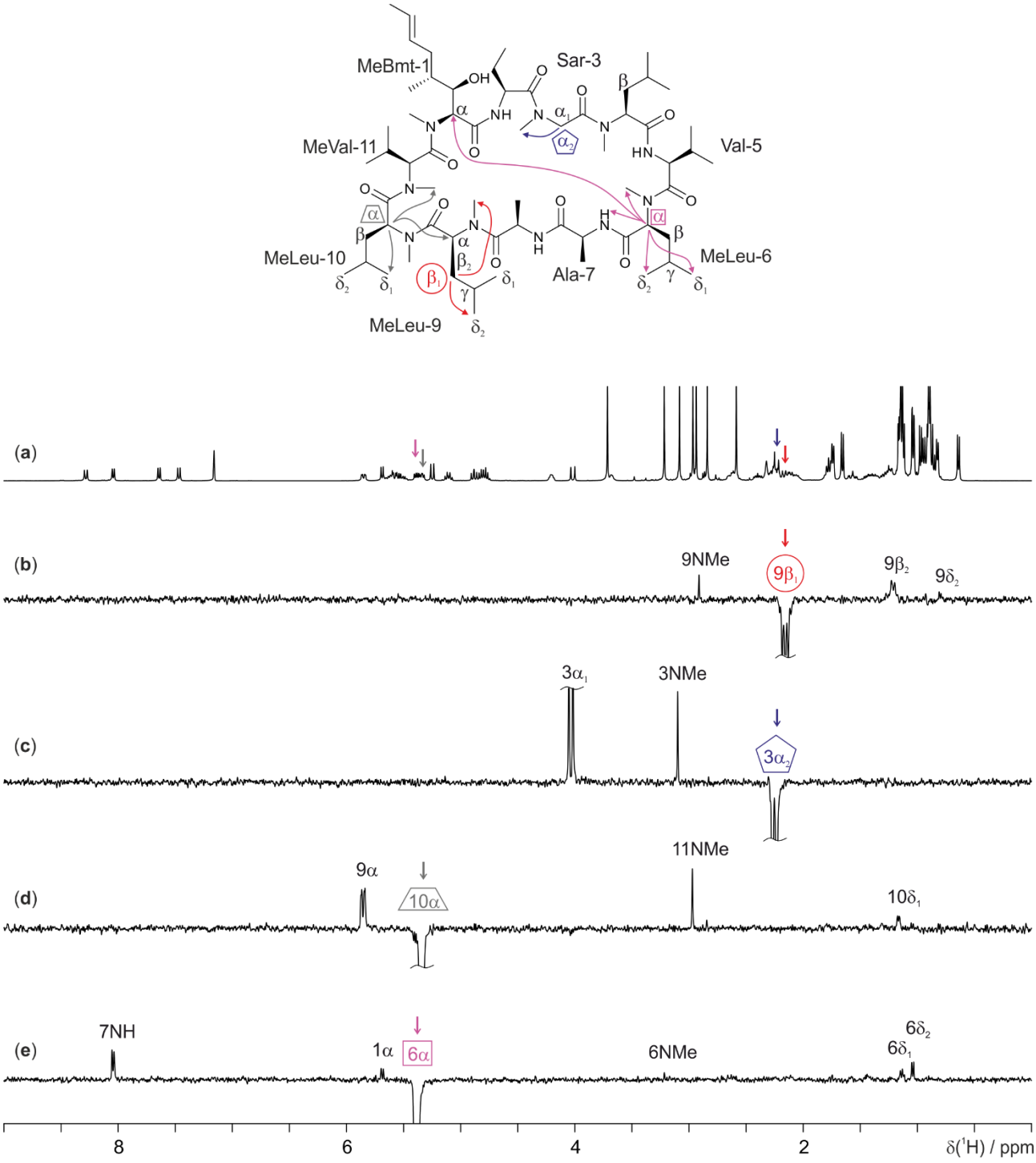

Figure S5: 400 MHz ^{1}H NMR spectra of 70 mM cyclosporin in C_6D_6. Molecular structure with relevant assignments[11] is shown above the spectra with arrows representing the ^{1}H-^{1}H ROEs observed; for simplicity, individual protons are not shown. (**a**) Conventional ^{1}H 1D spectrum. (**b-e**) GEMSTONE-ROESY spectra exciting at 2.18 (MeLeu-9β_1), 2.25 (Sar-3α_1), 5.34 (MeLeu-10α), and 5.39 (MeLeu-6α) ppm, respectively. All GEMSTONE parameters are provided in Table S2.

5. Signal phase and intensity anomalies

Occasionally, small phase and intensity anomalies are observed in the parent and/or ROE signals. Figure S6c shows that the components of the D -Ala-8β doublet have very small equal and opposite phase errors, leading to the net amplitude at the midpoint of the doublet being lower than expected. Such errors arise from the use of a hard spin echo to accommodate the refocusing CTP gradient G_1 (see Figure S1). Preliminary studies indicate that these anomalies are probably due to strong coupling. The nature of the phenomenon is still under investigation, but it is possible to minimise the impact of these anomalies if necessary by empirical adjustment of the hard echo timing. As the hard echo is required to achieve ultra-clean spectra, free of subtraction artefacts, it is not advisable to remove it unless subtraction artefacts are too small to be a problem. Both versions of the GEMSTONE-ROESY pulse sequence code (with and without the CTP gradients) are provided in Section D.

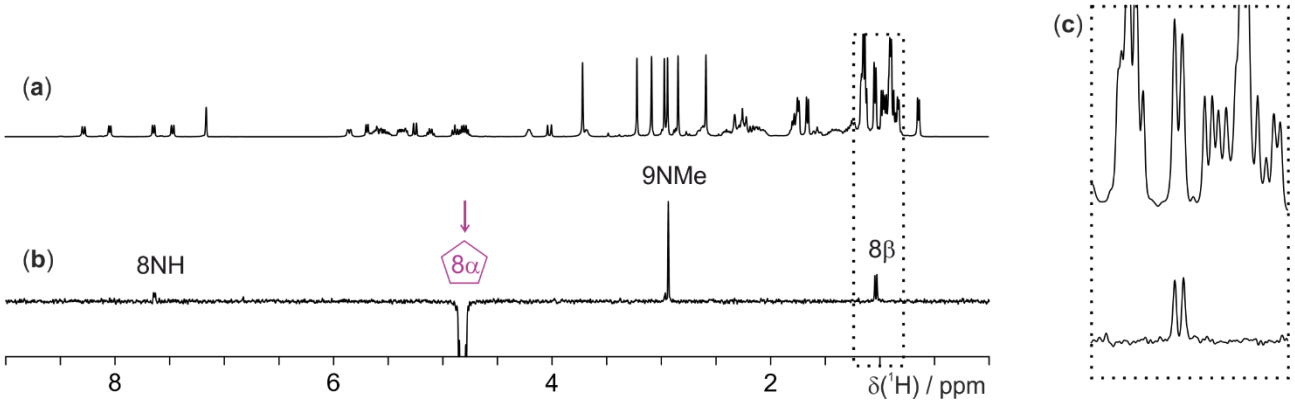

Figure S6: 400 MHz ^{1}H NMR spectra of 70 mM cyclosporin in C_6D_6. (**a**) Conventional ^{1}H 1D spectrum and (**b**) 1D GEMSTONE-ROESY spectrum selecting for the signal at 4.82 ppm (D-Ala-8α). The ROE signal at 1.04 ppm has been expanded in panel **c**.

C. Lacto-*N*-difucohexaose I

1. ^{1}H and pure shift NMR spectra of LNDFH I

Figure S7 shows the ^{1}H and PSYCHE pure shift NMR spectra of LNDFH I used to determine the exact chemical shifts of the signals to be targeted in the GEMSTONE experiments, highlighting the overlapping methyl doublets at approximately 1.25 ppm.

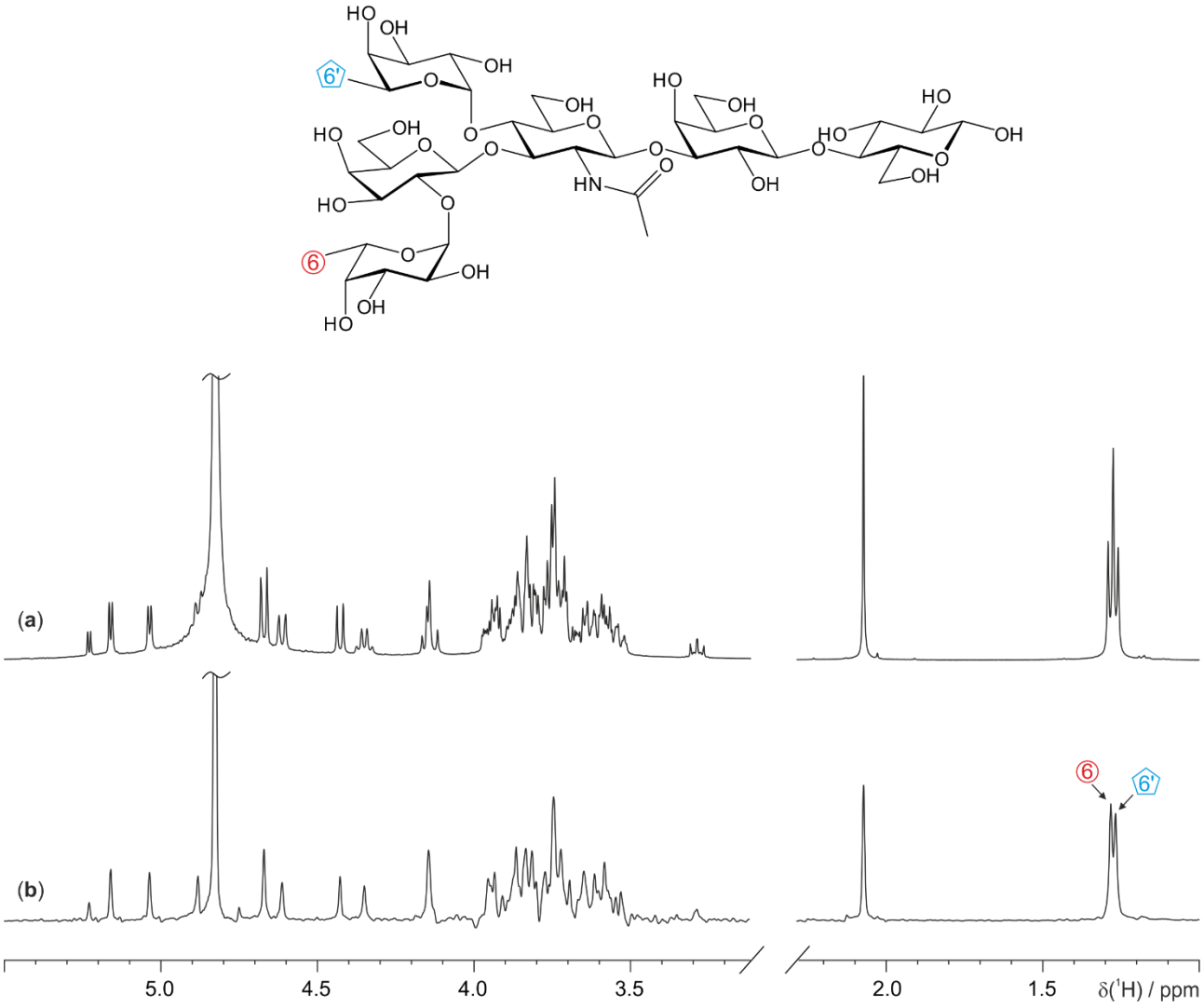

Figure S7: 400 MHz ^{1}H NMR spectra of 25 mM LNDFH I in D_2O. Molecular structure with relevant assignments is shown above the spectra. (**a**) Conventional ^{1}H 1D spectrum and (**b**) PSYCHE pure shift spectrum acquired using a spectral width of 5000 Hz (12.5 ppm), a chunk duration of 20 ms, and 20 chunks. A 30 ms double saltire pulse with a bandwidth of 10 kHz and a 20° flip angle was used with a simultaneous gradient of 0.53 G cm^{-1}. An AU processing macro, 'pshift' (freely available from https://www.nmr.chemistry.manchester.ac.uk/?q=node/372), was used to convert the pseudo-2D pure shift data into a 1D FID. Prior to Fourier transformation, zero-filling to 65536 complex points was applied, along with Gaussian weighting using a LB of –0.01 Hz and GB of 0. 002.

2. NOE *vs* ROE

Figure S7 shows a comparison of LNDFH I spectra obtained using GEMSTONE-NOESY (left) and GEMSTONE-ROESY (right). NOESY spectra provide some information on through-space interactions, but the NOEs are low in intensity, suggesting that the system is close to the NOE zero-crossing point. Low-intensity NOEs are difficult to observe and can be hidden by the noise. GEMSTONE-ROESY spectra provide clear, positive, ROEs which are greater in intensity than the corresponding NOEs.

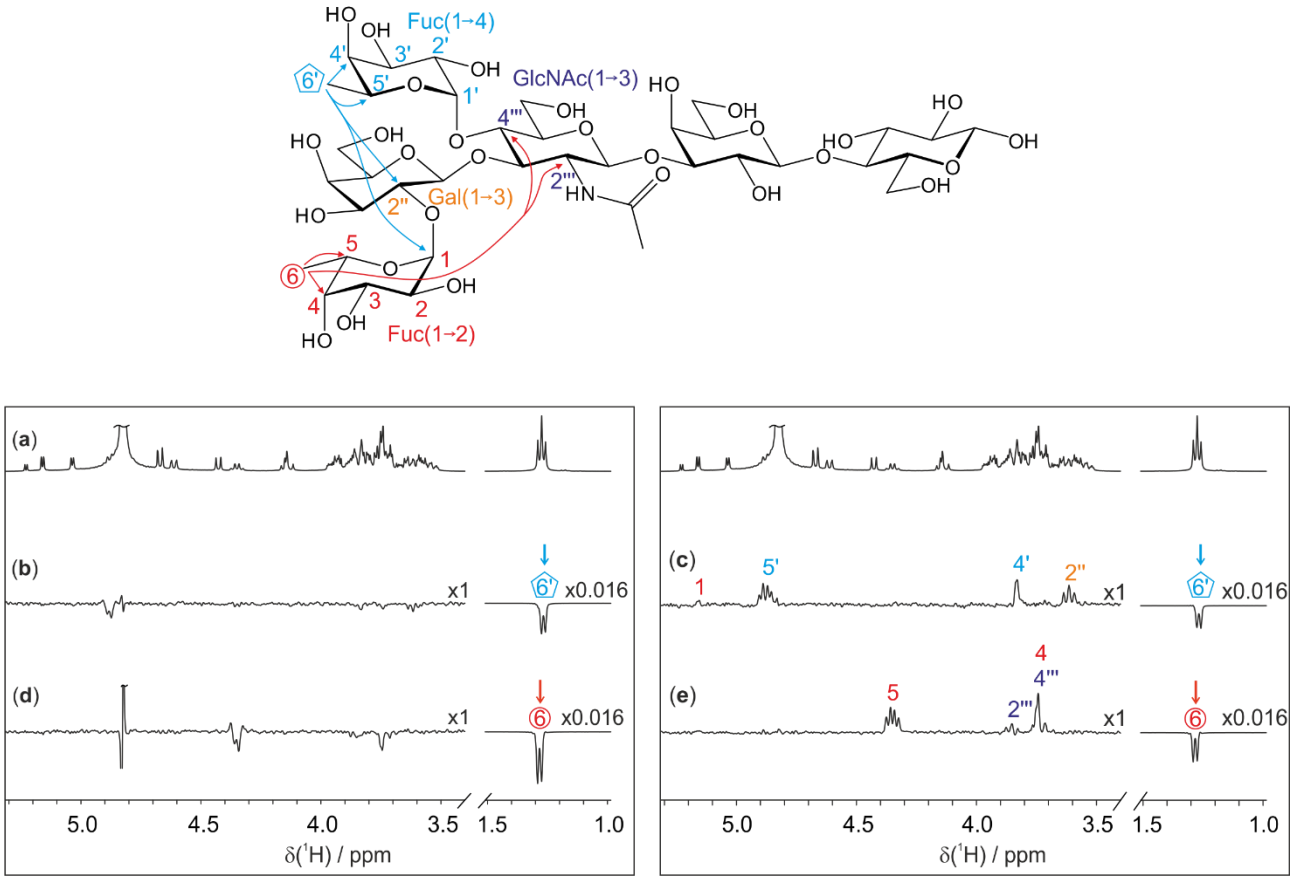

Figure S8: 400 MHz ^{1}H NMR spectra of 25 mM LNDFH I in D_2O. Molecular structure with relevant assignments is shown above the spectra with arrows representing the ^{1}H-^{1}H ROEs observed; for simplicity, individual protons are not shown. (**a**) Conventional ^{1}H 1D spectrum. (**b** and **d**) GEMSTONE-NOESY spectra selecting for signals at 1.27 (H6' fucose (1→4)) and 1.28 (H6 fucose (1→2)) ppm, respectively. The total NOESY mixing time was 400 ms. All other processing and acquisition parameters were kept consistent with the GEMSTONE-ROESY experiments. (**c** and **e**) GEMSTONE-ROESY spectra selecting for signals at 1.27 (H6' fucose (1→4)) and 1.28 (H6 fucose (1→2)) ppm, respectively. All GEMSTONE parameters are provided in Table S2. Other experimental parameters are provided in Section A.

D. Pulse program codes for Bruker spectrometer

1. GEMSTONE-ROESY with CTP gradient reinforcement

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;GEMSTONE-ROESY
;REFERENCES
;SPFGE selective (ROESY) experiment
;  J. Stonehouse, P. Adell, J, Keeler and A. J. Shaka:
;  J. Am. Chem. Soc., 116, 6037-6038 (1994)
;Symmetrization:
;  J. Schleucher, J. Quant, S. Glaser, C. Griesinger:
;  J. Magn. Reson A, 112, 144-151, 1995.
;EASY-ROESY sequence
;  C. M. Thiele, K. Petzold, J. Schleucher:
;  Chem. Eur. J. 15, 585-588, 2009.
; S. Boros, Gy Batta:
;  Magn. Reson. Chem. 2016
;Gradient selection:
;  J. Furrer:
;  J. Nat. Prod., 2009, 72, 14371441.
;Zero-quantum suppression:
;  M. J. Thrippleton, J. Keeler:
;  Angew. Chem. Int. Ed. 42, 3938-3941 (2003)
;GEMSTONE:
;  P. Kiraly, N. Kern, M. P. Plesniak, M. Nilsson, D. J. Procter, G. A. Morris, R. W. Adams:
;  Angew. Chem., Int. Ed. 2021, 60, 666-669.

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;$DIM=1D
;$TYPE=
;$SUBTYPE=
;$COMMENT=

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"p2=p1*2"
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"d11=30m+1s/(1+cnst54)"
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1u gron11
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1u groff
d17
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p16:gp1
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3u
p16:gp1*-1
d16 pl1:f1
d17
1u
1u gron12
(p42:sp42 ph6):f1
1u
1u groff
d17 pl1:f1
(p1 ph3):f1
3u
p17:gp2
d17
3u
(center (p25:sp25 ph0):f1 (p25:gp3))
d17
3u fq=cnst3(sfo hz):f1
(p61:sp24 ph0):f1
3u
(p62:sp16 ph0):f1
3u
(p61:sp26 ph0):f1
3u
3u fq=cnst4(sfo hz):f1
(p61:sp27 ph0):f1
3u
(p62:sp16 ph0):f1
3u
(p61:sp28 ph0):f1
3u
p17:gp4
d17 fq=cnst1(sfo hz):f1
3u
(center (p23:sp23 ph0):f1 (p23:gp5))
d17
3u pl1:f1
(p1 ph2):f1
DELTA
p2 ph0
20u
p16:gp6
d16
20u
go=2 ph31
30m mc #0 to 2 F0(zd)
50u LOCKH_OFF
exit
ph0=0
ph1=0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
ph2=0 2
ph3=0 0 2 2
ph4=0 0 0 0 1 1 1 1 2 2 2 2 3 3 3 3
ph5=0
ph6=0
ph31=0 2 2 0 2 0 0 2 0 2 2 0 2 0 0 2 2 0 0 2 0 2 2 0 2 0 0 2 0 2 2 0
;PULSES
;p1: f1 channel -  90 degree high power pulse
;pl1: f1 channel - power level for pulse (default)
;p12: f1 channel - 180 degree shaped pulse
;sp2: selective 180 shaped pulse
;spnam2: Rsnob.1000
;p12: f1 channel - 180 degree shaped pulse
;p16: defocus/refocus gradient pulse
;p17: gradient pulse [1 msec]
;SPINLOCK
;p15: ROESY mixing time
;p61: 1 ms, f1 channel - adiabatic ramps for spinlock
;p62: p15/2
;sp16: rectangular pulse shape for spinlock
;spnam16: Squa100.1000
;sp24: adiabatic ramp to negative offset spinlock
;spnam24: Gaussramp+down.1
;sp26: adiabatic ramp down from negative offset spinlock
;spnam26: Gaussramp+up.1
;sp27: adiabatic ramp up to positive offset spinlock
;spnam27: Gaussramp-down.1
;sp28: adiabatic ramp down from positive offset spinlock
;spnam28: Gaussramp-up.1
;ZERO-QUANTUM SUPRESSION
;p25: 50 ms duration of first ZQS element
;spnam25: Crp_60kHz_50ms
;sp25: power level for first ZQS element
;p23: 30 ms duration of second ZQS element
```

;spnam23: Crp_60kHz_30ms
;sp23: power level for the second ZQS element
;p41: adiabatic pulse
;p42: reverse sweep adiabatic pulse
;DELAYS
;d1: relaxation delay; 1-5 * T1
;d16: delay grad recovery for spinecho [200us]
;d17: delay for gradient recovery [1ms]
;WAVEMAKER GEMSTONE
;cnst50: band-width of the band-selective RSNOB pulse [Hz]
;cnst51: sweep-width of the adiabatic pulse [Hz]
;cnst52: duration of the adiabatic pulse [t1max/2: 30-150 ms]
;sp2(p12):wvm:eg_user:f1 userA1(cnst50 Hz) ss=10.0us;
;sp41:wvm:eg_wurst_1:f1 wurst-80(cnst51 Hz, cnst52 ms; L2H, Q=11) ss=5.0us;
;sp42:wvm:eg_wurst_2:f1 wurst-80(cnst51 Hz, cnst52 ms; H2L, Q=11) ss=5.0us;
;WAVEMAKER ZQS elements
;cnst53: bandwidth of first ZQS [kHz]
;cnst54: duration of first ZQS [ms]
;cnst55: bandwidth of second ZQS [kHz]
;cnst56: duration of second ZQS [ms]
;sp25(p25):wvm:ZQS1:f1 sm_chirp(cnst53 kHz, cnst54 ms; NPOINTS=10000, L2H, Q=11) ss=5.0us
;sp23(p23):wvm:ZQS2:f1 sm_chirp(cnst55 kHz, cnst56 ms; NPOINTS=10000, L2H, Q=11) ss=5.0us
;CONSTANTS
;cnst1: set RF pulse on-resonance
;cnst2: field strength of spin lock in Hz
;cnst30: conversion of spinlock angle into radians
;cnst31: Theta for the off resonance, Magic angle or 60 deg suggested
;EXTRA INFORMATION
;NS: 8 * n, total number of scans: NS * TD0
;DS: 4
;GRADIENTS
;gpz1: defocusing gradient
;gpz2: 31 % homospoil gradient
;gpz3: ~9.5% for ZQS elements
;gpz4: 46 % homospoil gradient
;gpz5: ~9% for ZQS elements
;gpz6: refocusing gradient [2*gp1]
;gpz11: +2.15%
;gpz12: -2.15%

;gpnam1: SMSQ10.100
;gpnam2: SMSQ10.100
;gpnam3: RECT.1
;gpnam4: SMSQ10.100
;gpnam5: RECT.1
;gpnam6: SMSQ10.100

2. GEMSTONE-ROESY without CTP gradient reinforcement

```
;GEMSTONE-ROESY
;REFERENCES
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;$CLASS=HighRes
;$DIM=1D
;$TYPE=
;$SUBTYPE=
;$COMMENT=

#include <Avance.incl>
#include <Grad.incl>
#include <Delay.incl>

;CONSTANTS
"cnst1=0"
"cnst30=tan((cnst31/180)*3.1416)"
"cnst3=(cnst2/cnst30)"
"cnst4=-(cnst2/cnst30)"
;PULSES
"p62=0.5*p15"
;DELAYS
"d11=30m"
"d11=30m+1s/(1+cnst50)"
"d11=30m+1s/(1+cnst51)"
"d11=30m+1s/(1+cnst52)"
"d11=30m+1s/(1+cnst53)"
"d11=30m+1s/(1+cnst54)"
"d11=30m+1s/(1+cnst55)"
"d11=30m+1s/(1+cnst56)"
"d11=30m"
;POWER for ROESY spin-lock
"p30=1000000.0/(cnst2*4)"
"cnst41= (p30/p1) * (p30/p1)"
"spw24=plw1/cnst41"
"spw16=plw1/cnst41"
"spw26=plw1/cnst41"
"spw27=plw1/cnst41"
"spw28=plw1/cnst41"
;ACQUISITION
"acqt0=0.0"


1 ze
2 30m
50u BLKGRAMP
50u LOCKH_OFF
  d1 pl1:f1
50u LOCKH_ON
  50u UNBLKGRAMP
  (p1 ph1):f1
d16
  1u
  1u gron11
  (p41:sp41 ph5):f1
  1u
1u groff
d16
  3u
  p16:gp1
  d16
  p12:sp2:f1 ph4
  3u
  p16:gp1
  d16 pl1:f1
```

```
 d16

  1u
  1u gron12
  (p42:sp42 ph6):f1
  1u
1u groff
d16 pl1:f1
  (p1 ph3):f1
3u
  p16:gp2
  d16
  3u
  (center (p25:sp25 ph0):f1 (p25:gp3))
  d16
  3u fq=cnst3(sfo hz):f1
  (p61:sp24 ph0):f1
  3u
  (p62:sp16 ph0):f1
  3u
  (p61:sp26 ph0):f1
  3u
  3u fq=cnst4(sfo hz):f1
  (p61:sp27 ph0):f1
  3u
  (p62:sp16 ph0):f1
  3u
  (p61:sp28 ph0):f1
  3u
  p16:gp4
  d16 fq=cnst1(sfo hz):f1
  (center (p23:sp23 ph0):f1 (p23:gp5))
  d16
  3u pl1:f1
  (p1 ph2):f1
                20u BLKGRAD
  go=2 ph31
  30m mc #0 to 2 F0(zd)

                50u LOCKH_OFF
  exit

ph0=0
ph1=0 2
ph2=0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
ph3=0 0 2 2
ph4=0 0 0 0 1 1 1 1 2 2 2 2 3 3 3 3
ph5=0
ph6=0
ph31=0 2 2 0 2 0 0 2 0 2 2 0 2 0 0 2 2 0 0 2 0 2 2 0 2 0 0 2 0 2 2 0

;PULSES
;p1: f1 channel -  90 degree high power pulse
;pl1: f1 channel - power level for pulse (default)
;p12: f1 channel - 180 degree shaped pulse
;sp2: selective 180 shaped pulse
;spnam2: Rsnob.1000
;p12: f1 channel - 180 degree shaped pulse
;p16: defocus/refocus gradient pulse
;p17: gradient pulse [1 msec]
;SPINLOCK
;p15: ROESY mixing time
;p61: 1 ms, f1 channel - adiabatic ramps for spinlock
;p62: p15/2
;sp16: rectangular pulse shape for spinlock
;spnam16: Squa100.1000
;sp24: adiabatic ramp to negative offset spinlock
;spnam24: Gaussramp+down.1
;sp26: adiabatic ramp down from negative offset spinlock
;spnam26: Gaussramp+up.1
;sp27: adiabatic ramp up to positive offset spinlock
;spnam27: Gaussramp-down.1
;sp28: adiabatic ramp down from positive offset spinlock
;spnam28: Gaussramp-up.1
;ZERO-QUANTUM SUPRESSION
;p25: 50 ms duration of first ZQS element
;spnam25: Crp_60kHz_50ms
;sp25: power level for first ZQS element
;p23: 30 ms duration of second ZQS element
;spnam23: Crp_60kHz_30ms
;sp23: power level for the second ZQS element
;p41: adiabatic pulse
;p42: reverse sweep adiabatic pulse
;DELAYS
;d1: relaxation delay; 1-5 * T1
;d16: delay grad recovery for spinecho [200us]
```

```
;d17: delay for gradient recovery [1ms]
;WAVEMAKER GEMSTONE
;cnst50: band-width of the band-selective RSNOB pulse [Hz]
;cnst51: sweep-width of the adiabatic pulse [Hz]
;cnst52: duration of the adiabatic pulse [t1max/2: 30-100 ms]
;sp2(p12):wvm:eg_user:f1 userA1(cnst50 Hz) ss=10.0us;
;sp41:wvm:eg_wurst_1:f1 wurst-80(cnst51 Hz, cnst52 ms; L2H, Q=11) ss=5.0us;
;sp42:wvm:eg_wurst_2:f1 wurst-80(cnst51 Hz, cnst52 ms; H2L, Q=11) ss=5.0us;
;WAVEMAKER ZQS elements
;cnst53: bandwidth of first ZQS [kHz]
;cnst54: duration of first ZQS [ms]
;cnst55: bandwidth of second ZQS [kHz]
;cnst56: duration of second ZQS [ms]
;sp25(p25):wvm:ZQS1:f1 sm_chirp(cnst53 kHz, cnst54 ms; NPOINTS=10000, L2H, Q=11) ss=5.0us
;sp23(p23):wvm:ZQS2:f1 sm_chirp(cnst55 kHz, cnst56 ms; NPOINTS=10000, L2H, Q=11) ss=5.0us
;CONSTANTS
;cnst1: set RF pulse on-resonance
;cnst2: field strength of spin lock in Hz
;cnst30: conversion of spinlock angle into radians
;cnst31: Theta for the off resonance, Magic angle or 60 deg suggested
;EXTRA INFORMATION
;NS: 8 * n, total number of scans: NS * TD0
;DS: 4
;GRADIENTS
;gpz1: 15 % gradient for selective pulse
;gpz2: 31 % homospoil gradient
;gpz3: ~9.5% for ZQS elements
;gpz4: 46 % homospoil gradient
;gpz5: ~9% for ZQS elements
;gpz11: +2.15%
;gpz12: -2.15%
;gpnam1: SMSQ10.100
;gpnam2: SMSQ10.100
;gpnam3: RECT.1
;gpnam4: SMSQ10.100
;gpnam5: RECT.1
```

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