Parallel NMR Supersequences: 10 Spectra in a Single Measurement

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Dedicated to Professor Ray Freeman on the occasion of his 90th birthday

(Supporting Information)
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1. Experimental Details.

Structure of molecules studied in this work are shown in Fig. S1. The tetrasaccharides were explored as 30 and 48 mM solutions in D$_2$O; the cyclosporine A sample was 50 mM in benzene-d$_6$; and the andrographolide sample was 40 mM in DMSO-d$_6$. The spectra were recorded on Bruker AVIII and NEO spectrometers operating at 600, 700 and 800 MHz $^1$H frequencies. Both room-temperature (TXI) and cryogenic (TCI) probes were used. Sensitivity permitting, all experiments can be recorded with a single scan per increment (see e.g., Fig. S13 in section 8a). Further experimental details are given in the corresponding figure captions.

All raw data of the NOAH experiments were processed with a single command (au-program) splitx_au. The latter along with the pulse programs and parameter files of the NOAH experiments described in this work are available from the Bruker User Library (https://www.bruker.com/en/services/bruker-user-library.html).

Pulse programs containing arbitrary combinations of NOAH modules may also be accessed from a new website called GENESIS (manuscript in preparation). The latest version of this may be accessed at https://nmr-genesis.co.uk/, but in order to guarantee the reproducibility of these instructions, please specifically use version 2.0.14: this is available at https://nmr-genesis.co.uk/2/0/14. To obtain the $p$-NOAH pulse sequences, turn on the “developer mode” switch near the top-right. Scroll down past the coloured module boxes and proceed directly to the “choose your own” text box. The pulse programs may be obtained by entering a series of module codes separated by spaces:

| Module Code | Description |
|-------------|-------------|
| C_HMBC_CF | HMBC with double F1 resolution |
| C_HMBC_CF_K | HMBC with twice the number of scans |
| C_HMBC_CFDD | Two interleaved HMBC spectra with different $^1$JCH evolution delays |
| C_HSQCC_DIA | HSQC-COSY |
| C_HSQCC_CIA | HSQC-CLIP-COSY |
| C_HSQCT_IA | HSQC-TOCSY |
| C_SEHSQC_IA | IPAP-seHSQC |
| C_SEHSQCJ | J-seHSQC with double F1 resolution |
| C_SEHSQCJ_K | J-seHSQC with twice the number of scans |

Note that these codes are not exhaustive; there is a complete list of modules linked in the FAQ section of the website. A few concrete examples of supersequences are as follows:

- to get $p$-NOAH-5 ($BS^5S^5T/S$) (Fig. 2), enter
  C_HMBC_CF_K C_HSQCC_DIA C_SEHSQCJ H_TOCSY
  [Note that this uses 2×NS for the HMBC and 2×resolution for the TOCSY, as described in the main text. This can be customized as desired according to the table above.]
- to get $p$-NOAH-6 ($BS^5S^5T/SS^5S$) (Fig. S5), enter
  C_HMBC_CF C_HSQCC_DIA C_SEHSQC_IA H_TOCSY
- to get $p$-NOAH-8 ($BS^5S^5/C/BSS^5S$) (Fig. S7), enter
  C_HMBC_CFDD C_HSQCC_DIA C_SEHSQC_IA H_TT_DM
- to get $p$-NOAH-10 ($BS^5S^5CR/BSS^5S$) (Fig. S13), enter
  C_HMBC_CFDD C_HSQCC_CIA C_SEHSQC_IA H_TT_CR
Fig. S1. Test molecules studied in this work.
2. HSQC-COSY modules

For the supersequences demonstrated in this work, we performed several comparisons of different implementations of the HSQC-COSY module. All of these replace the final reverse INEPT element in the standard HSQC (blue box, Fig. S2a) with an element which combines the $J_{CH}$ refocusing provided by the INEPT block with H–H coherence transfer. Three different versions were investigated, namely:

1) a double spin echo (DSE) version (Fig. S2b), denoted by $S^C$ elsewhere in this work;
2) the HSQC-CLIP-COSY (Fig. S2c) as reported by Gyöngyösi et al., [S1] denoted by $S^{Cc}$ elsewhere in this work;
3) a triple spin echo (TSE) version (Fig. S2d).

![Fig. S2](image)

**Fig. S2.** (a) HSQC module with reverse INEPT highlighted in blue. In all sequences, $\Delta$ is set to $1/(4 \cdot J_{CH})$, and $\tau$ delay for $^2H_H$ evolution is set to $1/(4 \cdot \sum J_{HH})$. (b–d) Coherence transfer elements for the various HSQC-COSY modules investigated in this work: (b) the “double spin echo” / “DSE” version (denoted by $S^C$); (c) the HSQC-CLIP-COSY module [S1] (denoted by $S^{Cc}$); (d) the “triple spin echo” / “TSE” version (not used in this work). The dotted $^{13}C$ 180° pulse is applied only on alternate scans to suppress spurious peaks arising from multistep $^1H$–$^1H$ coherence transfer.

Fig. S2 compares the three pulse sequences in the context of a NOAH-3 $S^S$SC$^C$ (HSQC-COSY + HSQC + CLIP-COSY) [S4] supersequence. This allows us to judge not only the quality of the HSQC-COSY spectrum itself, but also its effects on later modules in NOAH experiments. Of the three, the DSE version provides the greatest sensitivity (Fig. S3f), although the resulting lineshapes are a mixture of in-phase absorption + antiphase dispersion (inset, Fig. S3b). The CLIP version has lower sensitivity because the z-filter removes dispersion-mode contributions to the final signal (Fig. S3e); in return, the resulting lineshapes are in pure absorption-mode (inset, Fig. S3a). The TSE version has the same mixed lineshapes as the DSE version (insets, Fig. S3, c–d), but successfully preserves both bulk magnetisation (of protons not coupled to $^{13}C$) (Fig. S3, o–p), and additionally preserves any unused $^{13}C$–$^1H$ magnetisation that is not excited during the INEPT block, in a similar way to the previously described HSQC-TOCSY module [S2] (Fig. S3, k–l). Both the CLIP and DSE versions dephase unused magnetisation, causing the later modules to have decreased sensitivity.
Fig. S3. Comparisons of the CLIP, DSE, and TSE HSQC-COSY implementations. The TSE version is shown twice, once with \( f = 1 \) (i.e. using all of the \(^{13}\)C-bound proton magnetisation), and once with \( f = 0.8 \) (i.e. using only 80% of the \(^{13}\)C-bound proton magnetisation, leaving an additional 20% for the next module) [S2]. All spectra are taken from NOAH-3 HSQC-COSY / HSQC / CLIP-COSY supersequences. (a)–(d): The 2D HSQC-COSY spectra, including an inset showing peak lineshapes more clearly: only the CLIP version provides pure absorption-mode peaks. (e)–(h): Positive projections of the HSQC-COSY spectra: the DSE version has the greatest sensitivity. (i)–(l): Positive projections of the HSQC module: sensitivity is boosted only when using the TSE version with \( f < 1 \), in which a portion of \(^{13}\)C-bound proton magnetisation is preserved for use in the HSQC. (m)–(p): Positive projections of the CLIP-COSY module: only the TSE version properly preserves bulk magnetisation for this final homonuclear module (the value of \( f \) has no effect on this). Spectra were recorded on a 700 MHz Avance III spectrometer, equipped with a TCI H/C/N cryoprobe; the sample is 40 mM andrographolide in DMSO-\( d_6 \). The delays \( \Delta \) and \( \tau \) were set to 1.72 ms and 8.33 ms respectively.

The HSQC-COSY spectra shown in Fig. S3 are recorded using the pulse sequences shown in Fig. S2: in all of these, the final spin echo allows for evolution of \( J_\text{CH} \) for a total duration of \( 4\Delta = 1/J \). Thus, the base HSQC peaks (correlations between \(^{13}\)C and directly bonded \(^1\)H) are inverted, whereas the relayed HSQC-COSY peaks (correlations between \(^{13}\)C and \(^1\)H separated by two or three bonds) are not, resulting in the “antiphase” presentation seen in Fig. S3 (a–d). The “in-phase” presentation, where both sets of peaks have the same sign, is recorded by removing the final \(^{13}\)C 180° pulse in Fig. S2 (b–d). In the interleaved supersequences shown in this work, the two datasets have been added and subtracted to yield new datasets which only have one type of peak in them: these datasets are labelled as “HSQC-COSY” and “HSQC” in Figs. S4–S14.

In the actual supersequences shown in this work, the HSQC-COSY module is always preceded by the HMBC module, which already dephases bulk magnetisation. Furthermore, the HSQC which follows is sensitivity-enhanced, [S2, S3] which makes the magnetisation preservation in the TSE version slightly less important. Consequently, we have opted to use the DSE and CLIP versions in this work. However, in other scenarios the TSE version may prove to be more useful: for example, if the HMBC module is not used, or if it is desirable to boost the sensitivity of the seHSQC or lower sensitivity homonuclear modules (e.g. NOESY / ROESY) which follow it. p-NOAH pulse programmes containing...
the TSE version may be created via the GENESIS website (SI Section 1) using the C_HSQCC_IA module code in place of the other HSQC-COSY codes.

3. Structure elucidation of tetrasaccharide βLNNOMe from p-NOAH-5 (BS5S'T/S) spectra using the CASPER program

Full spectra of tetrasaccharides recorded using the p-NOAH-5 BS5S'T/S supersequence include the responses from the Me and CO groups and are shown in Fig. S4. The spectra were used for structure elucidation of βLNNOMe using the CASPER program. The latter correctly predicted the structure of this molecule from the p-NOAH-5 spectra. A comparison of the experimental and the predicted C–H correlation maps are shown in Fig. S4. A complete CASPER report is provided in section 11.

**Fig. S4.** Full spectra of βLNNOMe (30 mM in D2O) recorded with the p-NOAH-5 (BS5S'T/S) supersequence. The spectra were recorded in 36 min 29 sec with two scans per t1 increment on a Bruker NEO spectrometer operating at 700 MHz. The raw data matrix size of the individual spectra was 512 × 256 data points; this was extended to 1k × 1k by zero filling. The J-evolution delays were calculated assuming \( J_{CH} = 158 \) Hz and \( J_{CH} = 8 \) Hz, the TOCSY (DIPSI-2) mixing time was 80 ms, and the recovery delay \( (d_1) \) was 1.3 s. The spectral width was 2.5 kHz \((^1H)\) and 19.5 kHz \((^{13}C)\). The CO cross-peak in \( F_1 \) of the HMBC spectrum is folded into an empty spectral region at ca 65 ppm \((^{13}C)\).
4. *p*-NOAH-6 experiments

An example of spectra produced by the *p*-NOAH-6 (BS\textsuperscript{CST/SSAP}) supersequence is shown in Fig. S5. The top panel shows the schematic representation of the supersequence that was used to record the corresponding spectra shown below. Both the conventional and sensitivity-enhanced (se) versions of the HSQC pulse schemes were tested as the time-shared (TS) multiplicity edited IPAP-HSQC modules in position 3. The seHSQC version is preferred for sensitivity reasons. In principle, the in-phase (IP) and antiphase (AP) HSQC spectra may also be recorded as interleaved or sequential modules; however, the sensitivity advantages of the TS method would be lost. The IPAP-seHSQC spectra are shown before the final processing step that calculates a sum and a difference of the two data sets. Note that the HSQC-COSY spectrum contains only the (C–H–H (COSY) correlations that are separated from the C–H (HSQC) correlations.

![Diagram of the supersequence](image)

**Fig. S5.** Schematic representation of the *p*-NOAH-6 (BS\textsuperscript{CST/SSAP}) supersequence (top panel) introduced in this work. Below are the corresponding spectra of the tetrasaccharide stachyose (48 mM in D\textsubscript{2}O) recorded with this supersequence (X = COSY). The spectra were recorded in 19 min 39 sec on a Bruker NEO spectrometer operating at 600 MHz and equipped with the TXI room-temperature probe. The *J*-evolution delays were calculated assuming *J*\textsubscript{CH} = 158 Hz and *J*\textsubscript{CH} = 8 Hz; spectral width was 2.0 kHz (\textsuperscript{1}H) and 8 kHz (\textsuperscript{13}C), raw data size was 512 × 1024 data points where the data size of individual TS modules S\textsuperscript{Y/S} and S\textsuperscript{Y/S} was 512 × 128 × 2 data points and 512 × 256 data points for the TOCSY and HMBC modules recorded with double resolution; two scans per increment, TOCSY (DIPSI-2) mixing time τ\textsubscript{m} = 80 ms, recovery delay *d*\textsubscript{1} = 1.3 s. Positive peaks are shown in blue, negative peaks in red and magnitude mode spectra in black.
5. *p*-NOAH-7 experiments

An example of spectra produced by the *p*-NOAH-7 (BS\(^{S^{P}T}/BSS^{A}\)) supersequence is shown in Fig. S6. The top panel shows the schematic representation of the supersequence that was used to record the corresponding spectra shown below. A second HMBC module is added to the NOAH-6 supersequence shown in the previous section to allow recording of two HMBC spectra with different settings of the \(J(CH)\) evolution delay in the same experiment. The TOCSY module in position 4 is recorded with double resolution. Alternatively, two TOCSY spectra with different mixing times and a single HMBC spectrum with either double resolution or double number of scans may be recorded in a similar *p*-NOAH-7 scheme.

![Diagram of supersequence](image)

**Fig. S6.** Spectra of the tetrasaccharide stachyose (48 mM in D\(_2\)O) recorded with the *p*-NOAH-7 (BS\(^{S^{P}T}/BSS^{A}\)) supersequence (top panel) involving interleaved HMBC (B/B), TS HSQC-COSY/HSQC (S\(^{S}/S\)), multiplicity edited TS IPAP-seHSQC (S\(^{P}/S^{A}\)) and TOCSY (T) module. The experiment was recorded on a Bruker NEO spectrometer operating at 600 MHz and equipped with a TXI room temperature probe. The total duration of the experiment was 18 min 52 sec. The \(J\)-evolution delays were calculated assuming \(J_{CH} = 158\) Hz and \(\Delta J_{CH} = 8\) and 15 Hz, TOCSY (DIPSI-2) mixing time \(\tau_m = 80\) ms; spectral width was 2.0 kHz (\(^1\)H) and 8 kHz (\(^{13}\)C) and data matrix size of the individual spectra was 512 × 128 data points; two scans per increment, recovery delay, \(d_1 = 1.3\) s. The IPAP-seHSQC spectra are shown before the final processing step that calculates a sum and a difference of the two data sets. Positive peaks are shown in blue, negative peaks in red and magnitude mode spectra in black.
6. *p*-NOAH-8 experiments

This section describes several *p*-NOAH-8 supersequences demonstrating the flexibility of the proposed parallel NOAH experiments. We show that various modules can be easily replaced producing a variety of parallel NOAH supersequences.

a) *p*-NOAH-8 \((BS^SIP^T/BSS^AP^T)\)

An example of spectra produced by the *p*-NOAH-8 \((BS^SIP^T/BSS^AP^T)\) supersequence is shown in Fig. S7. The top panel shows the schematic representation of the supersequence that was used to record the corresponding spectra shown below. A second TOCSY module is added to the *p*-NOAH-7 supersequence shown in the previous section to allow recording of two TOCSY spectra with two different mixing times in the same experiment. The sensitivity enhanced HSQC pulse scheme was used in the time-shared IPAP-seHSQC module. The IPAP spectra are shown following a complete processing of the data that separates the high-field (top) and low-field (bottom) components of the *J*-coupled spectrum.

![Diagram of supersequence](image)

**Fig. S7.** Spectra of the tetrascarhide stachyose (48 mM in D_2O) recorded with the *p*-NOAH-8 \((BS^SIP^T/BSS^AP^T)\) supersequence (Fig. 1d in the main text) involving two interleaved HMBC modules \((B/B)\) with different *J*-evolution periods assuming *J*\(_{CH}\) of 10 and 5 Hz, TS HSQC-COSY/HSQC \((S^S/S)\), multiplicity edited TS IPAP-seHSQC \((S^P/S^P)\) and two interleaved TOCSY modules \((T/T)\) with mixing times of 10 and 80 ms. The spectra were recorded in 15 min 33 sec on a Bruker NEO spectrometer operating at 600 MHz and equipped with a TXI room temperature probe. The *J*-evolution delays were calculated assuming *J*\(_{CH}\) = 158 Hz and *J*\(_{CH}\) = 8 and 15 Hz; spectral width was 2.0 kHz \((^1H)\) and 10.1 kHz \((^{13}C)\), total data matrix size was 512 \(\times\) 1k data points \((512 \times 128\) per spectrum\)); two scans per increment, recovery delay, \(d_i = 1\) s. Complete processing of the multiplicity edited TS IPAP-seHSQC data separates the high-field (top) and low-field (bottom) components of the spectrum. Positive peaks are shown in blue, negative peaks in red and magnitude mode spectra are in black.
b) $\rho$-NOAH-8 ($\text{BS}^{\text{S}^{\text{p}}/\text{C}}/\text{BSS}^{\text{APT}}$)

An example of spectra produced by the $\rho$-NOAH-8 ($\text{BS}^{\text{S}^{\text{p}}/\text{C}}/\text{BSS}^{\text{APT}}$) supersequence is shown in Fig. S8. The top panel shows the schematic representation of the supersequence that was used to record the corresponding spectra shown below. In this experiment the first TOCSY module (short mixing time) introduced in the previous section is replaced with the CLIP-COSY pulse scheme that provides the same information, except more selectively. Complete processing of the multiplicity edited $\text{TS IPAP-seHSQC}$ data separates the high-field (top) and low-field (bottom) components of the spectrum.

**Fig. S8.** Spectra of the tetrasaccharide stachyose (48 mM in D$_2$O) recorded with the $\rho$-NOAH-8 ($\text{BS}^{\text{S}^{\text{p}}/\text{C}}/\text{BSS}^{\text{APT}}$) supersequence (top panel, $X = \text{COSY}$) involving two interleaved HMBC modules (B/B) with different $J$-evolution periods assuming $J_{\text{CH}}$ of 10 and 5 Hz, $\text{TS HSQC-COSY/HSQC (S}^5/\text{S)}$, multiplicity edited $\text{TS IPAP-seHSQC (S}^5/\text{S}^{\text{p}}$) and interleaved CLIP-COSY and TOCSY module (C$^5$T). The spectra were recorded in 34 min 22 sec on a Bruker NEO spectrometer operating at 600 MHz using 16 dummy scans and two scans per $t_1$ increment; the total data matrix size was 512 $\times$ 2k (512 $\times$ 256 per spectrum). The $J$-evolution delays were calculated assuming $J_{\text{CH}} = 158$ Hz and $J_{\text{CH}} = 8$ and 15 Hz, TOCSY (DIPSI-2) mixing time $\tau_m = 80$ ms, recovery delay $d_1 = 1.3$ s; spectral width was 3.25 kHz ($^1\text{H}$) and 8 kHz ($^{13}\text{C}$). The multiplicity edited $\text{TS IPAP-seHSQC}$ data are shown following a complete data processing that separates the high-field (top) and low-field (bottom) components of the spectrum. Positive peaks are shown in blue, negative peaks in red and magnitude mode spectra in black.
c) \( p\)-NOAH-8 (BS\(^7\)SC\(^3\)C/BSS\(^{APT}\))

An example of spectra produced by the \( p\)-NOAH-8 (BS\(^7\)SC\(^3\)C/BSS\(^{APT}\)) supersequence is shown in Fig. S9. The top panel shows the schematic representation of the supersequence that was used to record the corresponding spectra shown below. The supersequence is similar to the NOAH-8 experiment described in the previous section (Fig. S8), except the TS HSQC-COSY (S\(^7\)/S) module is replaced with the TS HSQC-TOCSY pulse scheme (S\(^7\)/S). The multiplicity edited IPAP-seHSQC spectra are shown following the final processing step that calculates a sum and a difference of the two data sets.

![Diagram](image)

**Fig. S9.** Spectra of the tetrasaccharide stachyose (48 mM in D\(_2\)O) recorded with the \( p\)-NOAH-8 (BS\(^7\)SC\(^3\)C/BSS\(^{APT}\)) supersequence (top panel, X = TOCSY) involving two interleaved HMBC modules (B/B) with different \( J\)-evolution periods, TS HSQC-TOCSY/HSQC (S\(^7\)/S), multiplicity edited TS IPAP-seHSQC (S\(^7\)/S\(^8\)) and interleaved CLIP-COSY and TOCSY module (C/T). The spectra were recorded in 19 min 15 sec on a Bruker NEO spectrometer operating at 600 MHz. The \( J\)-evolution delays were calculated assuming \( ^3J_{CH} = 158\) Hz and \( ^4J_{CH} = 8\) and 15 Hz; TOCSY (DIPSI-2) mixing times were 17 ms in HSQC-TOCSY and 80 ms in TOCSY module, recovery delay, \( d_1 = 1.3\) s, spectral width was 2 kHz (\( ^1\)H) and 8 kHz (\( ^13\)C), raw data matrix size was 512 \( \times \) 1024 data points (512 \( \times \) 128 per spectrum); two scans per increment, 16 dummy scans. Positive peaks are shown in blue, negative peaks in red and magnitude mode spectra in black.

d) \( p\)-NOAH-8 (BS\(^{CCc}\)SC\(^{IPc}\)C/BSS\(^{APT}\))

An example of spectra of a cyclic peptide, cyclosporin A (11 amino acids) recorded with the \( p\)-NOAH-8 (BS\(^{CCc}\)SC\(^{IPc}\)C/BSS\(^{APT}\)) supersequence is shown in Fig. S10. The top panel shows the schematic representation of the supersequence that was used to record the corresponding spectra shown below. The supersequence is similar to the NOAH-8 experiment described in the previous section (Fig. S9), except the HSQC-COSY (S\(^3\)) module is replaced with the HSQC-CLIP-COSY pulse scheme (S\(^3\)/S\(^2\)). The multiplicity edited IPAP-seHSQC spectra are shown before the final processing step that calculates a sum and a difference of the two data sets.
Fig. S10. Spectra of cyclosporin A (50 mM in benzene-d$_6$) recorded with the p-NOAH-8 (BS$^{13}$C$^{1}$C/BSS$^{13}$T) supersequence (top panel, X = CLIP-COSY) involving two interleaved HMBC modules (B/B) with different J-evolution periods, TS HSQC-CLIP-COSY/HSQC (S$^{13}$/S), multiplicity edited TS IPAP-seHSQC (S$^{13}$/S$^{13}$) and interleaved CLIP-COSY and TOCSY module (C$^{13}$/T). The spectra were recorded in 17 min 37 sec on a Bruker NEO spectrometer operating at 600 MHz. The J-evolution delays were calculated assuming $J_{CH} = 145$ Hz and $J_{CH} = 5$ Hz (spectrum 1) and 10 Hz (spectrum 2); TOCSY (DIPSI-2) mixing time $\tau_m = 80$ ms, recovery delay $d_1 = 1.3$ s, spectral width was 6.25 kHz ($^1$H) and 32 kHz ($^{13}$C), total data matrix size was 1024 $\times$ 1024 data points (1024 $\times$ 128 per spectrum); two scans per increment, 16 dummy scans. Positive peaks are shown in blue, negative peaks in red and magnitude mode spectra in black.
e) p-NOAH-8 (BS\textsuperscript{cc}S\textsuperscript{ip}R/BSS\textsuperscript{ap}T)

An example of spectra of cyclosporin A recorded with the p-NOAH-8 (BS\textsuperscript{cc}S\textsuperscript{ip}R/BSS\textsuperscript{ap}T) supersequence is shown in Fig. S11. The top panel shows the schematic representation of the supersequence that was used to record the corresponding spectra shown below. The supersequence is similar to the p-NOAH-8 experiment described in the previous section (Fig. S10), except the CLIP-COSY (C) module in position 4 is replaced with the ROESY (R) pulse scheme. The multiplicity edited IPAP-seHSQC spectra are shown before the final processing step that calculates a sum and a difference of the two data sets.

![Diagram of supersequence](image)

**Fig. S11.** Spectra of cyclosporin A (50 mM in benzene-\textit{d}_6) recorded with the p-NOAH-8 (BS\textsuperscript{cc}S\textsuperscript{ip}R/BSS\textsuperscript{ap}T) supersequence (top panel, X = CLIP-COSY) involving two interleaved HMBC modules (B/B) with different J-evolution periods, TS HSQC-CLIP-COSY/HSQC (S\textsuperscript{cc}/S), multiplicity edited TS IPAP-seHSQC (S\textsuperscript{ap}/S) and interleaved ROESY and TOCSY module (R/T). The spectra were recorded in 18 min 33 sec on a Bruker NEO spectrometer operating at 600 MHz. The J-evolution delays were calculated assuming \( J_{\text{CH}} = 145 \) Hz and \( J_{\text{CH}} = 5 \) Hz (spectrum 1) and 10 Hz (spectrum 2); TOCSY (DIPSI-2) mixing time \( t_m = 60 \) ms, recovery delay \( d_1 = 1.3 \) s, spectral width was 6.25 kHz (\textit{1}H) and 32 kHz (\textit{13}C), raw data matrix size was 1024 \times 1024 data points (1024 \times 128 per spectrum); two scans per increment, 16 dummy scans. Positive peaks are shown in blue, negative peaks in red and magnitude mode spectra in black.
7. *p*-NOAH-9 supersequences

An example of spectra of cyclosporin A recorded with the *p*-NOAH-9 (BS<sup>SC</sup>SIPCR/SS<sup>AP</sup>TGr) supersequence is shown in Fig. S12. The top panel shows the schematic representation of the supersequence that was used to record the corresponding spectra shown below. The supersequence is obtained from the *p*-NOAH-8 experiment described in the previous section 6e (Fig. S11), by replacing the ROESY (R) module with sequential COSY + ROESY pulse scheme [55] in the first of the two parallel supersequences. Likewise, in the other (second) parallel supersequence the TOCSY module is replaced with a pair of sequential TOCSY modules [55] recorded with different mixing times. The multiplicity edited IPAP-seHSQC spectra are shown following the final processing step that calculates a sum and a difference of the two data sets that separates the high-field and low-field components of the $^{1}J_{CH}$ doublet in the two spectra.

![Diagram of supersequence](image)

**Fig. S12.** Spectra of cyclosporin-A (50 mM in benzene-$d_{6}$) recorded with the *p*-NOAH-9 (BS<sup>SC</sup>SIPCR/SS<sup>AP</sup>TGr) supersequence on Bruker NEO spectrometer operating at 600 MHz and equipped with a TXI room temperature probe. The HMBC spectrum is recorded with double resolution the inset showing an expansion of the crowded region marked with a dotted rectangle. The TOCSY spectra were recorded with (1) 17 ms and (2) 60 ms DIPSY-2 mixing. The ROESY spectrum was recorded with 132 ms adiabatic off-resonance mixing. The $J$-evolution delays were calculated assuming $^{1}J(CH) = 145$ Hz and $^{4}J(CH) = 8$ Hz, recovery delay, $d_{r} = 1.3$ s, spectral width was 6.25 kHz ($^{1}$H) and 32 kHz ($^{13}$C), raw data matrix size was 1024 x 1280 data points (1024 x 128 per spectrum, except HMBC recorded with 256 increments), two scans per increment. The total experiment time was 18 min 49 s. Positive peaks are shown in blue, negative peaks in red and magnitude mode spectra are in black.
8. \textit{p-NOAH-10 supersequences}

\textit{a) p-NOAH-10 (BS\textsuperscript{C\textsubscript{c}S\textsubscript{IP}TT}/BSS\textsuperscript{AP}CR)}

Sensitivity permitting the experiments discussed in this work can be recorded with a single scan per $t_1$ increment. This shown in Fig. S13 using the p-NOAH-10 (BS\textsuperscript{C\textsubscript{c}S\textsubscript{IP}TT}/BSS\textsuperscript{AP}CR) pulse scheme described in more detail in the main text. The multiplicity edited IPAP-seHSQC spectra are shown before the final processing step that calculates a sum and a difference of the two data sets.

**Fig. S13.** Spectra of cyclosporin A (50 mM in benzene-$d_6$) recorded with a single scan per increment using the p-NOAH-10 (BS\textsuperscript{C\textsubscript{c}S\textsubscript{CR}/BSS\textsuperscript{AP}TT) supersequence consisting of two interleaved HMBC modules (B/B) with different J-evolution periods, TS HSQC-CLIP-COSY/HSQC (S\textsuperscript{C\textsubscript{c}}/S), multiplicity edited TS IPAP-seHSQC (S\textsuperscript{IP}/S\textsuperscript{AP}) and sequential COSY and ROESY modules (CR) interleaved with two sequential TOCSY modules (TT). The spectra were recorded in 9 min 34 sec on a Bruker NEO spectrometer operating at 600 MHz and equipped with a room-temperature TXI probe. The J-evolution delays were calculated assuming $\nu_{CH} = 145$ Hz and $\nu_{CH} = 5$ Hz (spectrum 1) and 10 Hz (spectrum 2), TOCSY mixing times were 17 and 60 ms, ROESY mixing time was 132 ms; spectral width was 6.25 kHz ($^1$H) and 32 kHz ($^{13}$C), the total data matrix size was 1024 x 1280 data points (1024 x 128 per spectrum); one scan per increment, 16 dummy scans, recovery delay $d_1 = 1.3$ s. Positive peaks are shown in blue, negative peaks in red and magnitude mode spectra in black.
b) \textit{p-NOAH-10 (BS}^{S-CN/BSS}^{AP TT})

The \textit{p-NOAH-10 (BS}^{S-CN/BSS}^{AP TT}) supersequence is derived from the NOAH-10 scheme discussed in the previous section by replacing the ROESY module with a NOESY pulse scheme. The 10 spectra recorded with this supersequence are shown in Fig. S14. Both, positive and negative NOESY peaks are observed at 600 MHz and the peak intensities are generally very low. The ROESY module is preferred for this sample at 600 MHz (\textit{^1H}) frequency.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig_s14.png}
\caption{Spectra of cyclosporin A (50 mM in benzene-$d_6$) recorded with the \textit{p-NOAH-10 (BS}^{S-CN/BSS}^{AP TT}) supersequence (Fig 1e in the main text, except ROESY is replaced with NOESY) involving two interleaved HMBC modules (B/B) with different J-evolution periods assuming $J_{CH} = 10$ and 5 Hz, TS HSQC-CLIP-COSY/HSQC ($S^S/S^S$), TS IPAP-seHSQC ($S^S/S^S$) and sequential COSY and NOESY modules (CN) interleaved with two sequential TOCSY modules (TT) with mixing times of 17 and 60 ms. The spectra were recorded in 19 min 45 sec on a Bruker NEO spectrometer operating at 600 MHz. The J-evolution delays were calculated assuming $J_{CH} = 141.2$ Hz and $J_{CH} = 8$ Hz; spectral width was 6.25 kHz (\textit{^1H}) and 32 kHz (\textit{^13C}), data matrix size of the individual spectra was 1024 x 128 data points; two scans per increment, recovery delay $d_1 = 1.3$ s. Positive peaks are shown in blue, negative peaks in red and magnitude mode spectra in black.}
\end{figure}
9. Spectra recorded with NOAH-4 BSTT supersequence.

The spectra of a cyclic peptide, cyclosporin A (11 amino acids) recorded using the NOAH-4 BSTT supersequence are shown in Fig. S15. This supersequence was implemented, tested, and later used to design parallel NOAH experiments containing sequential TOCSY modules. Both echo–anticecho [S5] and States versions of the TOCSY modules were explored. As expected, there is a slight sensitivity advantage in the latter case which was then used in the parallel supersequences.

![Diagram of the supersequence](image)

**Fig. S15.** Spectra of cyclosporin A (50 mM in benzene-$d_6$) recorded with the NOAH-4 (BSTT) supersequence involving HMBC (B), multiplicity edited HSQC (S), and two sequential (States) TOCSY modules (TT). The spectra were recorded in 6 min 52 sec on a Bruker NEO spectrometer operating at 600 MHz. The $J$-evolution delays were calculated assuming $J_{CH} = 145$ Hz and $J_{CH} = 10$ Hz, TOCSY (DIPSI-2) mixing times were 10 and 60 ms, spectral width was 6.25 kHz ($^1$H) and 32 kHz ($^{13}$C), the raw data matrix size was $1024 \times 1024$ data points ($1024 \times 256$ per spectrum). The spectra were recorded with one scan per increment, 8 dummy scans, recovery delay, $d_1 = 1$ s. Positive peaks are shown in blue, negative peaks in red and magnitude mode spectra in black.

10. NOAH experiments in larger peptides

While it remains to be seen whether the new methodology will work for larger peptides or even small (intrinsically disordered) proteins, it is important to note that this technique is designed for samples with the natural abundance of isotopes.

11. References

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12. CASPER report for tetra-saccharide βLNnTOMe
\[ \beta\text{-LNnT-OMe} \]

Chemical Formula: $C_{27}H_{47}NO_{21}$
Molecular Weight: 721.7
NMR chemical shift data from NOAH-5 experiment on βLNNTomE as input to CASPER

|        |        |        |        |        |        |        |
|--------|--------|--------|--------|--------|--------|--------|
| 13C [ppm] | 1H [ppm] | [Hz]     | 13C [ppm] | 1H [ppm] | 13C [ppm] | 1H [ppm] |
| 103.51 | 4.71   | 160.3   | 103.51 | 3.81   | 103.51 | 3.81   |
| 103.64 | 4.48   | 159.5   | 103.64 | 3.55   | 103.64 | 3.55   |
| 103.72 | 4.44   | 162.2   | 103.72 | 3.59   | 103.84 | 3.58   |
| 103.84 | 4.41   | 162.0   | 103.94 | 3.30   | 103.84 | 3.30   |
| 69.07  | 4.16   | 73.52   | 4.41   | 82.79  | 4.71   | 4.48   | 3.93   |
| 60.82  | 3.99   | 71.69   | 4.48   | 79.10  | 4.44   | 4.48   | 3.55   |
| 60.61  | 3.96   | 70.70   | 4.44   | 78.93  | 4.48   | 4.44   | 4.16   |
| 69.30  | 3.93   | 55.95   | 4.71   | 57.96  | 4.41   | 4.44   | 3.72   |
| 55.95  | 3.81   | 4.48   | 3.67   |
| 60.82  | 3.80   | 4.41   | 3.61   |
| 61.68  | 3.80   | 4.41   | 3.30   |
| 61.77  | 3.78   |        |        |
| 78.93  | 3.74   |        |        |
| 72.94  | 3.74   |        |        |
| 61.68  | 3.74   |        |        |
| 76.11  | 3.74   |        |        |
| 82.79  | 3.72   |        |        |
| 75.64  | 3.72   |        |        |
| 73.26  | 3.67   |        |        |
| 75.12  | 3.65   |        |        |
| 79.10  | 3.63   |        |        |
| 75.50  | 3.61   |        |        |
| 70.70  | 3.60   |        |        |
| 75.35  | 3.59   |        |        |
| 57.96  | 3.58   |        |        |
| 71.69  | 3.55   |        |        |
| 73.52  | 3.30   |        |        |
| 22.93  | 2.04   |        |        |
b-LNnT-OMe

Calculated $^{13}$C and $^1$H chemical shifts

Structure

$\beta$-D-Gal$^{iv}(1 \rightarrow 4) \beta$-D-GlcNAc$^{iii}(1 \rightarrow 3) \beta$-D-Gal$^{ii}(1 \rightarrow 4) \beta$-D-GlcOMe$^{i}$

| $\rightarrow 4) \beta$-D-GlcOMe$^{i}$ | 1   | 2   | 3   | 4   | 5   | 6   | 6    | Me$_{1}$    |
|-------------------------------------|-----|-----|-----|-----|-----|-----|------|-------------|
| Expected Calc. Error: 0.00          | 103.95 | 73.71 | 75.34 | 79.69 | 75.64 | 61.24 | 57.91  |
|                                     | 4.40  | 3.33 | 3.66 | 3.64 | 3.60 | 3.82 | 3.99  | 3.58       |
| $\rightarrow 3) \beta$-D-Gal$^{ii}(1 \rightarrow 3$ | 1   | 2   | 3   | 4   | 5   | 6   | 6    |             |
| Expected Calc. Error: 0.00          | 103.81 | 70.92 | 82.81 | 69.10 | 75.76 | 61.75 |       |
|                                     | 4.46  | 3.63 | 3.73 | 4.14 | 3.72 | 3.78 | 3.80  |             |
| $\rightarrow 4) \beta$-D-GlcNAc$^{iii}(1 \rightarrow 3$ | 1   | 2   | 3   | 4   | 5   | 6   | 6    | Me$_{2}$  | CO$_{2}$   |
| Expected Calc. Error: 0.17          | 103.14 | 56.28 | 73.09 | 79.66 | 75.47 | 61.24 | 23.25  | 175.47     |
|                                     | 4.77  | 3.78 | 3.76 | 3.71 | 3.62 | 3.85 | 3.98  | 2.04       |
| $\beta$-D-Gal$^{iv}(1 \rightarrow 3$ | 1   | 2   | 3   | 4   | 5   | 6   | 6    |             |
| Expected Calc. Error: 0.17          | 103.80 | 71.89 | 73.55 | 69.48 | 76.18 | 61.80 |       |
|                                     | 4.48  | 3.57 | 3.67 | 3.95 | 3.73 | 3.77 | 3.79  |             |

Assignment of $^{13}$C, $^1$H resonances

| Experimental | Calculated | Expt-Calc | Assignment       |
|--------------|------------|-----------|------------------|
| 103.84 - 4.41 | 103.95 - 4.40 | 0.03     | $\beta$-D-GlcOMe$^{i}$ - 1 |
| 103.72 - 4.44 | 103.81 - 4.46 | 0.03     | $\beta$-D-Gal$^{ii}$ - 1 |
| 103.64 - 4.48 | 103.80 - 4.48 | 0.03     | $\beta$-D-Gal$^{iv}$ - 1 |
| 103.51 - 4.71 | 103.14 - 4.77 | 0.10     | $\beta$-D-GlcNAc$^{iii}$ - 1 |
| 82.79 - 3.72  | 82.81 - 3.73  | 0.01     | $\beta$-D-Gal$^{ii}$ - 3 |
| 79.10 - 3.63  | 79.69 - 3.64  | 0.12     | $\beta$-D-GlcOMe$^{i}$ - 4 |

Continued on next page
| Experimental | Calculated | Expt-Calc | Assignment          |
|--------------|------------|-----------|---------------------|
| 78.93 - 3.74 | 79.66 - 3.71 | 0.15      | β-D-GlcNAc<sub>iii</sub> - 4 |
| 76.11 - 3.74 | 76.18 - 3.73 | 0.02      | β-D-Gal<sub>iv</sub> - 5 |
| 75.64 - 3.72 | 75.76 - 3.72 | 0.02      | β-D-Gal<sub>ii</sub> - 5 |
| 75.50 - 3.61 | 75.64 - 3.60 | 0.03      | β-D-GlcOMe<sub>i</sub> - 5 |
| 75.35 - 3.59 | 75.47 - 3.62 | 0.04      | β-D-GlcNAc<sub>iii</sub> - 5 |
| 75.12 - 3.65 | 75.34 - 3.66 | 0.04      | β-D-GlcOMe<sub>i</sub> - 3 |
| 75.50 - 3.61 | 75.64 - 3.60 | 0.05      | β-D-GlcOMe<sub>i</sub> - 3 |
| 72.94 - 3.74 | 73.09 - 3.76 | 0.04      | β-D-GlcNAc<sub>iii</sub> - 3 |
| 71.69 - 3.55 | 71.89 - 3.57 | 0.04      | β-D-Gal<sub>iv</sub> - 2 |
| 70.70 - 3.60 | 70.92 - 3.63 | 0.05      | β-D-Gal<sub>ii</sub> - 2 |
| 69.30 - 3.93 | 69.48 - 3.95 | 0.04      | β-D-GlcOMe<sub>i</sub> - 4 |
| 69.07 - 4.16 | 69.10 - 4.14 | 0.02      | β-D-Gal<sub>ii</sub> - 4 |
| 61.77 - 3.78 | 61.80 - 3.79 | 0.01      | β-D-GlcOMe<sub>iv</sub> - 6 |
| 61.77 - 3.75 | 61.80 - 3.77 | 0.02      | β-D-GlcOMe<sub>iv</sub> - 6' |
| 61.68 - 3.80 | 61.75 - 3.80 | 0.01      | β-D-Gal<sub>ii</sub> - 6 |
| 61.68 - 3.74 | 61.75 - 3.78 | 0.04      | β-D-Gal<sub>ii</sub> - 6' |
| 60.82 - 3.99 | 61.24 - 3.99 | 0.08      | β-D-GlcOMe<sub>i</sub> - 6 |
| 60.61 - 3.96 | 61.24 - 3.98 | 0.13      | β-D-GlcNAc<sub>iii</sub> - 6 |
| 60.61 - 3.85 | 61.24 - 3.85 | 0.13      | β-D-GlcNAc<sub>iii</sub> - 6' |
| 60.82 - 3.80 | 61.24 - 3.82 | 0.09      | β-D-GlcOMe<sub>i</sub> - 6' |
| 57.96 - 3.58 | 57.91 - 3.58 | 0.01      | β-D-GlcOMe<sub>i</sub> - Me<sub>1</sub> |
| 55.95 - 3.81 | 56.28 - 3.78 | 0.07      | β-D-GlcNAc<sub>iii</sub> - 2 |
| 22.93 - 2.04 | 23.25 - 2.04 | 0.06      | β-D-GlcNAc<sub>iii</sub> - Me<sub>2</sub> |

Error=1.57 (0.05/signal), RMS error=0.06.

Assignment of $^1$H, $^1$H correlations

| Experimental | Calculated | Expt-Calc | Assignment          |
|--------------|------------|-----------|---------------------|
| 4.71 - 3.81  | 4.77 - 3.78 | 0.07      | β-D-GlcNAc<sub>iii</sub> - 1 - 2 |
| 4.71 - 3.74  | 4.77 - 3.76 | 0.06      | β-D-GlcNAc<sub>iii</sub> - 1 - 3 |
| 4.71 - 3.59  | 4.77 - 3.62 | 0.07      | β-D-GlcNAc<sub>iii</sub> - 1 - 5 |
| 4.48 - 3.93  | 4.48 - 3.95 | 0.02      | β-D-Gal<sub>iv</sub> - 1 - 4 |
| 4.48 - 3.67  | 4.48 - 3.67 | 0.00      | β-D-Gal<sub>iv</sub> - 1 - 3 |
| 4.48 - 3.55  | 4.48 - 3.57 | 0.02      | β-D-Gal<sub>iv</sub> - 1 - 2 |
| 4.44 - 4.16  | 4.46 - 4.14 | 0.03      | β-D-Gal<sub>ii</sub> - 1 - 4 |
| 4.44 - 3.72  | 4.46 - 3.72 | 0.02      | β-D-Gal<sub>ii</sub> - 1 - 5 |
| 4.44 - 3.60  | 4.46 - 3.63 | 0.03      | β-D-Gal<sub>ii</sub> - 1 - 2 |
| 4.41 - 3.65  | 4.40 - 3.64 | 0.02      | β-D-GlcOMe<sub>i</sub> - 1 - 4 |
| 4.41 - 3.61  | 4.40 - 3.60 | 0.02      | β-D-GlcOMe<sub>i</sub> - 1 - 5 |
| 4.41 - 3.30  | 4.40 - 3.33 | 0.02      | β-D-GlcOMe<sub>i</sub> - 1 - 2 |

Error=0.40 ppm (0.03 ppm/signal), RMS error=0.03 ppm.
Assignment of long range $^{13}$C, $^1$H correlations

| Experimental | Calculated | Expt-Calc | Assignment |
|--------------|------------|-----------|------------|
| 103.84 - 3.58 | 103.95 - 3.58 | 0.02 | $\beta$-D-GlcOMe$^i$ - 1, $\beta$-D-GlcOMe$^i$ - Me$_1$ |
| 103.84 - 3.30 | 103.95 - 3.33 | 0.04 | $\beta$-D-GlcOMe$^i$ - 1, $\beta$-D-GlcOMe$^i$ - 2 |
| 103.72 - 3.59 | 103.81 - 3.63 | 0.04 | $\beta$-D-Gal$^{ii}$ - 1, $\beta$-D-Gal$^{ii}$ - 2 |
| 103.64 - 3.55 | 103.80 - 3.57 | 0.04 | $\beta$-D-Gal$^{iv}$ - 1, $\beta$-D-Gal$^{iv}$ - 2 |
| 103.51 - 3.81 | 103.14 - 3.78 | 0.08 | $\beta$-D-GlcNAc$^{iii}$ - 1, $\beta$-D-GlcNAc$^{iii}$ - 2 |
| 82.79 - 4.71 | 82.81 - 4.77 | 0.06 | $\beta$-D-Gal$^{ii}$ - 3, $\beta$-D-GlcNAc$^{iii}$ - 1 |
| 79.10 - 4.44 | 79.69 - 4.46 | 0.12 | $\beta$-D-GlcOMe$^i$ - 4, $\beta$-D-Gal$^{ii}$ - 1 |
| 78.93 - 4.48 | 79.66 - 4.48 | 0.15 | $\beta$-D-GlcNAc$^{iii}$ - 4, $\beta$-D-Gal$^{iv}$ - 1 |
| 73.52 - 4.41 | 73.71 - 4.40 | 0.04 | $\beta$-D-GlcOMe$^i$ - 2, $\beta$-D-GlcOMe$^i$ - 1 |
| 71.69 - 4.48 | 71.89 - 4.48 | 0.04 | $\beta$-D-Gal$^{iv}$ - 2, $\beta$-D-Gal$^{iv}$ - 1 |
| 70.70 - 4.44 | 70.92 - 4.46 | 0.05 | $\beta$-D-Gal$^{iv}$ - 2, $\beta$-D-Gal$^{iv}$ - 1 |
| 57.96 - 4.41 | 57.91 - 4.40 | 0.02 | $\beta$-D-GlcOMe$^i$ - Me$_1$, $\beta$-D-GlcOMe$^i$ - 1 |
| 55.95 - 4.71 | 56.28 - 4.77 | 0.09 | $\beta$-D-GlcNAc$^{iii}$ - 2, $\beta$-D-GlcNAc$^{iii}$ - 1 |

Error=0.78 (0.06/signal), RMS error=0.05.

Assigned experimental $^{13}$C and $^1$H chemical shifts

Structure

$\beta$-D-Gal$^{iv}$ (1→4)$\beta$-D-GlcNAc$^{iii}$ (1→3)$\beta$-D-Gal$^{ii}$ (1→4)$\beta$-D-GlcOMe$^i$

$^{13}$C Error: 1.72. $^1$H Error: 0.09. 103.84 73.52 75.12 79.10 75.50 60.82 57.96

$\beta$-D-Gal$^{ii}$ (1→3)$\beta$-D-Gal$^{iv}$ (1→) $^{13}$C Error: 0.55. $^1$H Error: 0.12. 103.72 70.70 82.79 69.07 75.64 61.68

$^{13}$C Error: 2.65. $^1$H Error: 0.19. 103.51 55.95 72.94 78.93 75.35 60.61 22.93 n.d.

$\beta$-D-Gal$^{iv}$ (1→) $^{13}$C Error: 0.93. $^1$H Error: 0.08. 103.64 71.69 73.26 69.30 76.11 61.77

3