

Diversifying NMR Supersequences with New HSQC-Based Modules

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Abstract

The sensitivity-enhanced HSQC, as well as HSQC-TOCSY, experiments can be incorporated into NOAH (NMR by Ordered Acquisition using ¹H detection) supersequences. Importantly, these heteronuclear modules preserve the magnetisation required for subsequent acquisition of other homonuclear modules in the supersequence. With these new modules, we reach a total of over 600 practically applicable NOAH supersequences which yield high-quality 2D spectra with greatly reduced experiment durations.

In recent years, there has been significant interest in techniques which accelerate the acquisition of NMR data, especially for multidimensional spectra.^{1–3} Some of the more readily implemented approaches involve the use of multiple-FID acquisitions, using either single or multiple receivers. Of these, one of the most versatile approaches is to utilise different “pools” of magnetisation for the sequential collection of different spectra without an intervening recovery delay, as illustrated by the NOAH (NMR by Ordered Acquisition using ¹H detection) technique.⁴ Virtually all of the most common 2D experiments used in small molecule characterisation, such as HMBC, HSQC, COSY, NOESY, and TOCSY, can be concatenated in a modular fashion to form *supersequences* which collectively use only one recovery delay (d_1) (Figure 1a). As the recovery delay accounts for the large majority of experiment time in 2D NMR, the NOAH approach can provide time savings of up to $\sim 4\times$ compared to the conventional individual acquisition of each spectrum, in which each

constituent experiment would have its own recovery delay.

One-bond heteronuclear correlation experiments, namely HMQC and HSQC, play a central role in the structural elucidation of small organic molecules and biomolecules.⁵ These experiments are also a core component of many NOAH experiments, since the magnetisation they use (protons directly coupled to dilute nuclei, i.e. ¹³C or ¹⁵N) can be efficiently differentiated from the “bulk” magnetisation of protons that are not directly attached to these NMR-active nuclei (herein referred to as uncoupled protons).^{4d,6} At the same time, due to the low natural abundance of these heteronuclei, these spectra are typically less sensitive than the homonuclear spectra that are placed towards the end of the supersequence. Consequently, for dilute samples, the minimum experimental time is generally dictated by these heteronuclear experiments. Any improvements in experiment sensitivity can be translated into greater time savings.

In the 1990s, Cavanagh, Rance, and Kay introduced the sensitivity-enhanced HSQC (seHSQC) experiment, which improves on the sensitivity of an ordinary echo-antiecho HSQC by up to a factor of 2.⁷ This is accomplished by converting two magnetisation components that are cosine- and sine-modulated in t_1 into observable magnetisation prior to detection, in the so-called preservation of equivalent pathways (PEP) scheme. Here, we show how the original seHSQC sequence can be modified such that it can be used as a NOAH module. We add further diversification by introducing a HSQC-TOCSY module, derived from the ASAP-HSQC-TOCSY,⁸ that is also compatible with the NOAH strategy. Both of these modules can be inserted either independently or together into NOAH supersequences, allowing large amounts of chemical information to be acquired in short times.

The NOAH-4 MSCN experiment (Figure 1a) is an example of a NOAH supersequence, which yields ¹⁵N HMQC, ¹³C HSQC, COSY, and NOESY spectra in one single experiment. The implementation of this supersequence relies on the fact that the output of any one module contains all the necessary magnetisation components required for downstream modules. For example, both the standard NOAH HMQC (Figure 1b) and HSQC (Figure 1c) modules return the bulk magnetisation back to its equilibrium position (+z). In the MSCN sequence, this bulk magnetisation can therefore

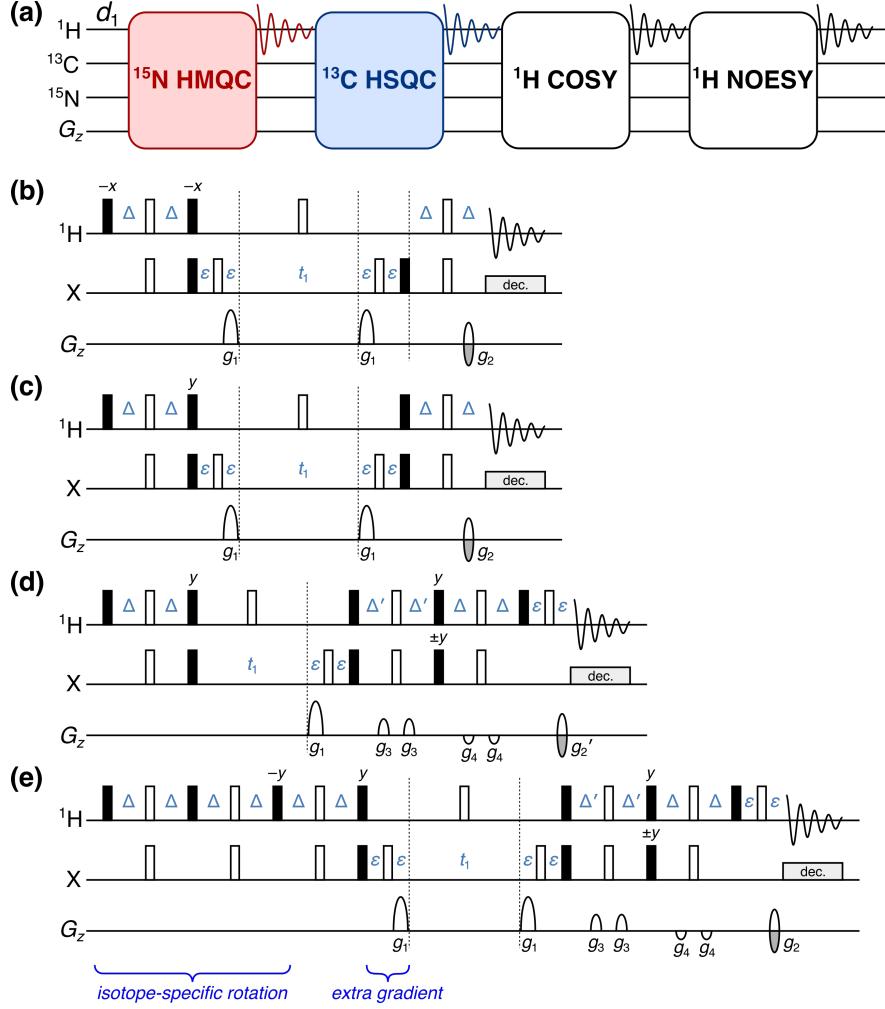


Figure 1: (a) Overview of a typical NOAH supersequence (MSCN, using the single-letter abbreviations previously defined^{4a}). The ¹⁵N–¹H HMQC and ¹³C–¹H HSQC modules are highlighted: these may be replaced with the new seHSQC module proposed in this work. (b) Original NOAH HMQC module,^{4a,9} abbreviated as “M”. (c) Original NOAH HSQC module without sensitivity enhancement,^{4a,10} abbreviated as “S”. (d) Cavanagh–Rance–Kay (CRK) seHSQC.⁷ (e) NOAH seHSQC module, abbreviated as “Sp” (this work). Filled and unfilled bars represent 90° and 180° pulses respectively; all 180° pulses on ¹³C are adiabatic (swept-frequency) pulses. All pulses are applied along +x unless otherwise noted. The delays are chosen as follows: $\Delta = 1/(4 \cdot ^1J_{\text{XH}})$, $\Delta' = 1/(8 \cdot ^1J_{\text{CH}})$ or $1/(4 \cdot ^1J_{\text{NH}})$, and ε is the minimum time needed for a gradient and subsequent recovery. All gradients are 1 ms long, except for g_1 and g_2 in ¹⁵N experiments which are 2.5 ms long. Gradient amplitudes, as percentages of maximum gradient strength, are as follows: $g_1 = 80\%$; $g_2 = \pm 40.2\%$ (¹³C) or $\pm 16.2\%$ (¹⁵N); $g_2' = g_2/2$; $g_3 = 11\%$; $g_4 = -5\%$. The signs of g_2 and g_2' are alternated in each t_1 increment to provide echo–antiecho selection. Refer to Figure S1 for product operator analysis. [Do we need to talk about phase cycling?]

be used as the input to the COSY and NOESY homonuclear modules which follow. However, the original Cavanagh–Rance–Kay (CRK) seHSQC (Figure 1d) does not obey this principle: it causes bulk magnetisation to be dephased by coherence transfer pathway (CTP) gradients. Consequently,

downstream modules can only utilise any bulk magnetisation that has relaxed during the HSQC FID acquisition, leading to drastic losses in signal intensity. This is illustrated using a NOAH-2 SpCc (seHSQC + CLIP-COSY¹¹) supersequence (Figure 2a): while the CRK seHSQC implementation afford significant sensitivity gains (primarily for CH peaks, as predicted by theory¹²), the COSY module which follows suffers from an almost complete loss of intensity.

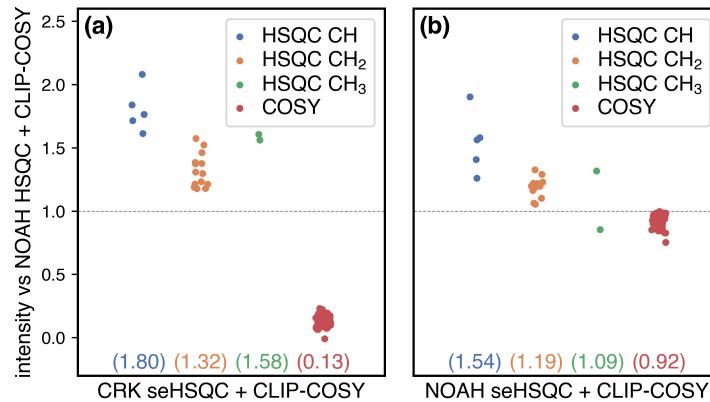


Figure 2: [Perhaps we could add spectra here? I think the graph is the most important piece of information, but I'm sure people like to see spectra more than graphs...] Sensitivity comparisons for NOAH-2 SpCc (seHSQC + CLIP-COSY) supersequences, using the CRK (Figure 1d) and NOAH (Figure 1e) seHSQC implementations. All intensities are normalised against the NOAH-2 SCc (HSQC + CLIP-COSY) supersequence, without HSQC sensitivity enhancement. HSQC intensities are further grouped by multiplicity. Numbers in parentheses indicate averages over all peaks of a given type. (a) Using the original CRK seHSQC. The CRK seHSQC does not preserve the bulk magnetisation, leading to severely reduced COSY intensities. (b) Using the NOAH seHSQC. Spectra were obtained on a 700 MHz Bruker AV III equipped with a TCI H/C/N cryoprobe; the sample used was 40 mM andrographolide in DMSO-*d*₆.

Our solution is based on the simple observation that the bulk magnetisation in the seHSQC will be returned to $+z$ if the phase of the initial ^1H 90° pulse is changed by 90° to $+y$. To generate the HSQC signal, however, the same pulse needs to be applied along $+x$. Overall, what is required is therefore a pulse sequence element which simultaneously acts as a 90_x° (or 90_{-x}°) pulse on protons coupled to ^{13}C , and as a 90_y° pulse on uncoupled protons. We accomplish this by prepending a double heteronuclear spin echo, which we refer to as the *isotope-specific rotation* (ISR), to the pulse sequence. It is similar to the *zz*-filter, which we have previously used in the NOAH HMBC module to retain the magnetisation of directly coupled protons for a subsequent HSQC module.^{4b,4d} However, the ISR has different pulse phases to this and consequently leads to a different overall

outcome. While the BIG-BIRD element developed by Briand and Sørensen¹³ is also capable of effecting this, we find that the ISR provides greater signal-to-noise in both the seHSQC itself, as well as downstream modules (Figure S5).

In addition to the ISR, the NOAH seHSQC module also contains a CTP gradient prior to the t_1 period (highlighted in Figure 1e). This gradient is not necessary for the seHSQC module itself, but instead serves to suppress artefacts in downstream modules, which would otherwise arise from bulk magnetisation that evolves during either half of the HSQC t_1 period. This then evolves again in the t_1 period of a later homonuclear module (e.g. COSY), resulting in each COSY peak with $\Omega_1 = \Omega_H$ being accompanied by a pair of “wing” artefacts at $\Omega_1 = \Omega_H \pm (\Omega_H \cdot SW_C)/(2 \cdot SW_H)$, which can reach $\sim 5\%$ of the intensity of their parent peaks. Importantly, the artefacts arising from diagonal peaks can have intensities that are comparable to genuine crosspeaks (Figure S6), which highlights the importance of suppressing these artefacts.

With these modifications, the NOAH seHSQC module provides clear sensitivity gains over the NOAH HSQC module, while preserving essentially the same amount of 1H magnetisation for downstream modules (Figure 2b). The modifications present in the NOAH seHSQC, particularly the ISR, mean that the sensitivity improvements are slightly lower as compared to the original CRK implementation. On average, CH and CH_2 peaks have $1.54\times$ and $1.19\times$ increased sensitivity respectively relative to the NOAH HSQC module. However, a dramatic improvement is seen in the COSY module which follows. In contrast to the CRK seHSQC, which completely destroys the requisite bulk magnetisation, the NOAH seHSQC preserves the majority of it, performing 92% as well as the original HSQC module.

Multiplicity editing can be easily incorporated into the NOAH seHSQC sequence (Figure S2), leading to similar sensitivity gains relative to the HSQC module. It is noteworthy that the original NOAH HSQC (without sensitivity enhancement) places the bulk magnetisation in the xy -plane during the t_1 and editing periods, whereas the newly proposed NOAH seHSQC places the bulk along $\pm z$. In the former, the bulk magnetisation is therefore subject to homonuclear coupling (J_{HH}) evolution, leading to a small decrease in the sensitivity of later homonuclear modules when

multiplicity editing is introduced. Since homonuclear experiments typically have a greater inherent sensitivity than the HSQC, this minor loss is not a problem, and is far outweighed by the benefits of incorporating multiplicity editing in the HSQC. Nevertheless, the fact that the seHSQC does not suffer from such a penalty is a welcome benefit. As a result, the edited NOAH seHSQC slightly outperforms the edited HSQC in terms of preserving bulk magnetisation, with subsequent homonuclear modules enjoying a slight boost in sensitivity (Figure S3).

[I'm a bit hesitant to talk about ASAP-seHSQC here especially given discoveries of last Friday...]

The proposed seHSQC module can be similarly implemented for ^{15}N experiments. Currently, in NOAH supersequences, $^{15}\text{N}-^1\text{H}$ correlations are primarily obtained using the HMQC module, since this manipulates bulk magnetisation more favourably than the HSQC (*vide infra*).^{4a,9} Compared to this, the new seHSQC module can provide greater than $4\times$ greater sensitivity (Figure 3). This arises partly because of the use of the PEP sensitivity enhancement scheme, in which the (reverse?) INEPT transfer delays Δ' can optimised for NH peaks. However, there is also a significant improvement due to the fact that peaks in the ^{15}N seHSQC are not broadened in the indirect dimension by J_{HH} , unlike in the HMQC. Although the seHSQC retains a slightly smaller amount of bulk magnetisation ($\sim 70\%$, versus $\sim 80\%$ for the HMQC), this is generally not a problem, since it is the ^{15}N module which typically has the lowest intrinsic sensitivity in a supersequence.

While a ^{15}N HSQC module (without sensitivity enhancement) would still benefit from multiplet collapse, it comes with other severe drawbacks. As previously discussed, the HSQC module places bulk magnetisation in the xy -plane during the t_1 period. Consequently, the amount of bulk magnetisation that is retained decreases as t_1 is lengthened, leading to line broadening in the indirect dimensions of all downstream modules (Figure S7). Whilst this is not a problem with the ^{13}C HSQC where typical ^{13}C indirect dimension acquisition times are relatively short, the smaller spectral widths in ^{15}N experiments can mean downstream modules suffer losses in both sensitivity and resolution. The seHSQC module avoids this issue entirely, making it especially well-suited to obtaining ^{15}N correlations.

For optimal performance, the ^{15}N seHSQC module benefits from one notable change with respect

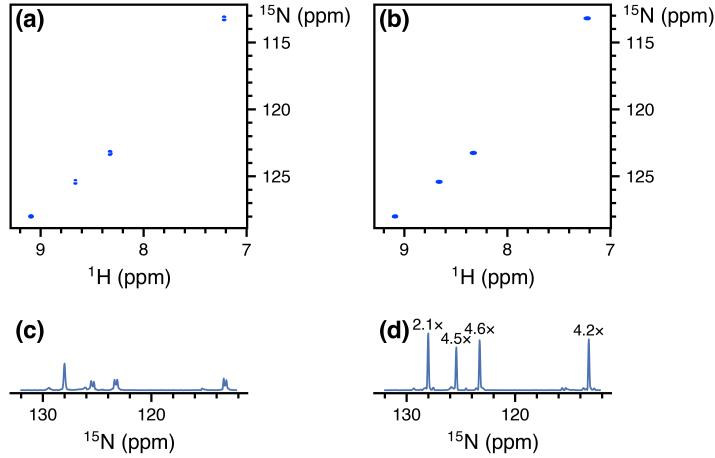


Figure 3: Comparison of the new ^{15}N - ^1H seHSQC with the standard NOAH HMQC module, taken from NOAH-3 XSpCc supersequences (^{15}N experiment + ^{13}C seHSQC + CLIP-COSY). (a) ^{15}N HMQC spectrum. (b) ^{15}N seHSQC spectrum. (c) Projection of HMQC onto the f_1 axis. Splitting due to J_{HH} is clearly visible for three of the four peaks. (d) Projection of seHSQC onto the f_1 axis. Signal-to-noise improvements relative to the HMQC spectrum are indicated over each peak. The largest gains are observed for peaks where the multiplet structure is collapsed; however, even in the absence of that, a $\sim 2\times$ gain is still obtained. Spectra were obtained on a 700 MHz Bruker AV III equipped with a TCI H/C/N cryoprobe; the sample used was 40 mM gramicidin in DMSO- d_6 .

to its ^{13}C counterpart, which is that the CTP gradients g_1 and g_2 (Figure 1e) are all lengthened from a typical duration of 1 ms to 2.5 ms. This is to more effectively dephase any residual bulk magnetisation that, due to the cumulative effect of pulse imperfections, is transverse just prior to detection. If left uncontrolled, this magnetisation can lead to significant levels of artefacts in the seHSQC module itself. The ^{13}C seHSQC does not need this gradient extension because of the larger amplitude of g_2 ; however, the corresponding ^{15}N gradient is weaker by a factor of $\gamma_{\text{C}}/\gamma_{\text{N}} \approx 2.5$, thus requiring a longer duration in order to produce comparable artefact attenuation. In practice, we find that gradient durations of 2 to 2.5 ms provide sufficient suppression whilst not causing any appreciable difference in the intensity of the desired crosspeaks (Figure S8).

In scenarios where high resolution in the ^{15}N dimension is not required, it can prove useful to reduce the number of t_1 increments and in its place increase the number of transients acquired.^{2b,14} In new versions of the NOAH pulse programmes (including those provided in the *Supporting Information*), this feature can be enabled by specifying a factor k by which to perform this scaling. Note that

the scaling is only applied to the ^{15}N module; all other modules are left untouched. In our hands, setting $k = 2$ or 4 for the ^{15}N HMQC can lead to significant sensitivity gains of up to $\sim 2\times$, since J_{HH} splitting in the indirect dimension can no longer be resolved (Figure S9). This point is not relevant to the seHSQC, and here k -scaling on its own has only a tiny effect on peak height (and signal-to-noise), since any sensitivity gained from the extra transients is offset by the broadening (Figure S10). However, the later t_1 increments which were not acquired can be reconstructed using linear projection¹⁵ to mitigate this line broadening. The resulting spectra display sensitivity gains of up to a factor of k , although the fidelity of the reconstruction can suffer for large k , particularly with the HMQC (Figures S11–S12).

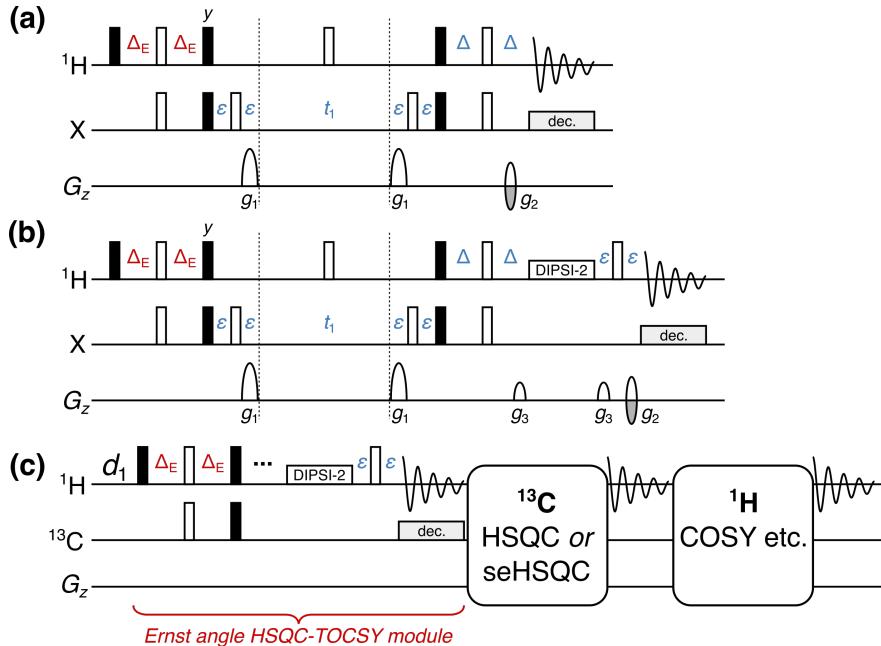


Figure 4: (a) NOAH HSQC module with modified INEPT delay $\Delta_E = (\sin^{-1} f)/(4 \cdot {}^1J_{\text{CH}})$, where f is the fraction of $^{13}\text{C}-{}^1\text{H}$ magnetisation excited. (b) NOAH HSQC-TOCSY module (“St”), modified from the ASAP-HSQC-TOCSY.⁸ The gradients g_3 are 1 ms long, and are set to 19% of the maximum gradient amplitude. (c) Overview of a NOAH-3 StSX or StSpX supersequence. The $^{13}\text{C}-{}^1\text{H}$ magnetisation is partly used by the initial HSQC-TOCSY module, with a subsequent HSQC or seHSQC using the remaining magnetisation. The bulk magnetisation is retained for one or more homonuclear modules at the end. All other symbols have the same meanings as in Figure 1.

Next, we note that the HSQC module (though not the new seHSQC module) allows an arbitrary amount of $^{13}\text{C}-{}^1\text{H}$ magnetisation to be excited, with the remainder returned to $+z$. In order to excite a proportion f of $^{13}\text{C}-{}^1\text{H}$ magnetisation ($0 < f \leq 1$), the initial INEPT delay must

be shortened by a factor of $\sin^{-1} f$. The remaining $(1 - f)$ of the magnetisation, plus any that recovers during the first HSQC FID, can then be used for a second HSQC module in the same supersequence. The collection of multiple HSQC spectra in one multi-FID acquisition (MFA) experiment has previously been accomplished by keeping the two CTPs in the CRK seHSQC separate, with the cosine- and sine-modulated CTPs each contributing to one spectrum.¹⁶ However, with a value of $f = 0.8$, the NOAH strategy already provides slightly higher sensitivity for both spectra. Furthermore, the sensitivity of the second HSQC can be further boosted by using the new seHSQC module in place of it (Figure S13).

By adding a period of isotropic mixing prior to detection, the NOAH HSQC module may be converted to a HSQC-TOCSY module. This is similar to the previously reported ASAP-HSQC-TOCSY,⁸ the key difference being that in the present NOAH context, unused $^{13}\text{C}-^1\text{H}$ and bulk magnetisation is preserved for use in other modules, instead of later t_1 increments as in the ASAP experiment. Compared to the existing MFA HSQC-TOCSY/HSQC experiment,^{16a} our approach has several characteristics which make it particularly amenable to use in NOAH supersequences. Firstly, the vast majority of bulk magnetisation is preserved, as required for homonuclear module(s) to be appended in a NOAH supersequence (in practice, losses of ca. 10% are observed due to pulse imperfections). In contrast, the MFA sequence, much like the original CRK seHSQC on which it is based, dephases bulk magnetisation and causes a 80–90% sensitivity loss in downstream spectra. Secondly, the sensitivity of both spectra in a NOAH experiment can be optimised through the value of f ; this allows a larger amount of $^{13}\text{C}-^1\text{H}$ magnetisation to be used for the inherently less sensitive HSQC-TOCSY (in our experience, setting $f = 0.9$ provides a good balance). Lastly, since each NOAH module is independently executed, the NOAH approach allows multiplicity editing to be enabled for only the HSQC and not the HSQC-TOCSY, where accidental overlap may lead to crosspeaks being lost unexpectedly.

Despite these benefits, we note that it is not possible to simply insert a TOCSY mixing block into the seHSQC module presented here, as that will lead to the bulk magnetisation being dephased. Therefore, the NOAH HSQC-TOCSY module will still have lower overall sensitivity than a conventional seHSQC-TOCSY, which can make use of the PEP scheme.

(I removed the bit on fast acquisition being most useful for concentrated samples. We can add back later if needed.)

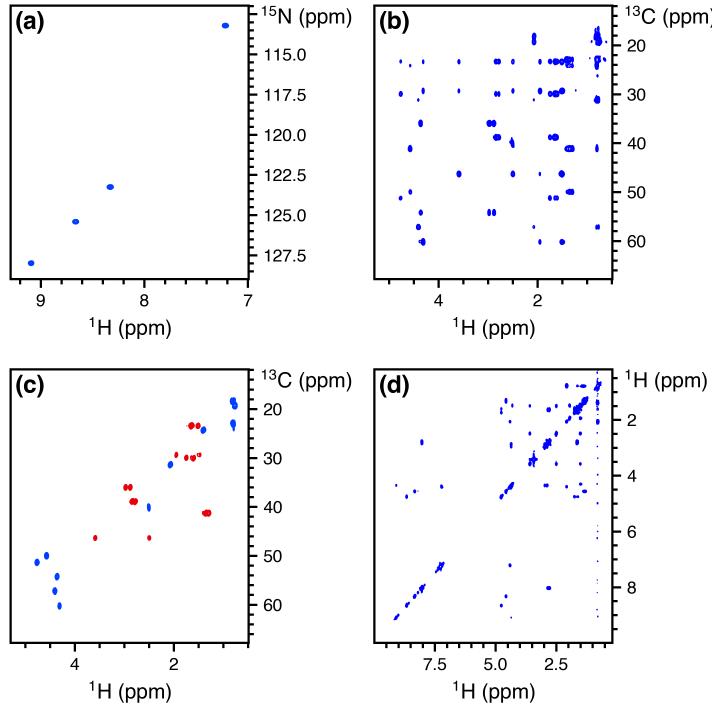


Figure 5: Example spectra obtained from the NOAH-4 SpnStSpCc supersequence. 256 t_1 increments were used, with 2 scans per increment. The total experiment time was 17 minutes and 35 seconds. (a) ¹⁵N seHSQC. (b) ¹³C HSQC-TOCSY (30 ms mixing, $f = 0.9$). (c) Multiplicity-edited ¹³C seHSQC. Notice that having the edited seHSQC removes the need for the less desirable HSQC-TOCSY editing. (d) CLIP-COSY. Spectra were obtained on a 700 MHz Bruker AV III equipped with a TCI H/C/N cryoprobe; the sample used was 40 mM gramicidin in DMSO-*d*₆.

The NOAH-4 SpnStSpCc (¹⁵N seHSQC, ¹³C HSQC-TOCSY, ¹³C seHSQC, and CLIP-COSY) supersequence is one of many ways in which the new modules discussed above can be included in practical experiments. The spectra thus obtained are shown in Figure 5. While individual collection of the four spectra above would require 57 minutes and 8 seconds, the NOAH-4 supersequence takes only 17 minutes and 35 seconds, which represents a 3.25× speedup. For typical organic molecules, new supersequences such as the NOAH-4 StSpCT allow the rapid and complete collection of C–H and H–H correlations (Figure S15). Experiment times can be further reduced through the use of non-uniform sampling (Figure S16), which is compatible with nearly all of the supersequences shown here. One can also prepend the NOAH HMBC module (“B”);^{4d} this uses the semi-adiabatic

zz-filter to preserve magnetisation of protons directly coupled to ^{13}C and ^{15}N heteronuclei, which is precisely the magnetisation required by the HSQC-based modules presented here (Figure S17).

The new seHSQC and HSQC-TOCSY implementations add to the preexisting diversity in NOAH modules, bringing the total number of plausible NOAH supersequences to over 600. The AU scripts needed for processing of these modules, as well as a number of the more commonly used pulse sequences, are provided in the *Supporting Information*; others are available upon request from the authors. However, a more user-friendly and customisable method for the generation of NOAH pulse sequences is clearly needed to handle the sheer variety currently available. Our work towards this will be reported in the near future.

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References

1. (a) Frydman, L.; Scherf, T.; Lupulescu, A. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 15858–15862; (b) Frydman, L.; Lupulescu, A.; Scherf, T. *J. Am. Chem. Soc.* **2003**, *125*, 9204–9217.
2. (a) Nolis, P.; Pérez-Trujillo, M.; Parella, T. *Angew. Chem. Int. Ed.* **2007**, *46*, 7495–7497; (b) Parella, T.; Nolis, P. *Concepts Magn. Reson.* **2010**, *36A*, 1–23.
3. (a) Kupče, Ě.; Freeman, R.; John, B. K. *J. Am. Chem. Soc.* **2006**, *128*, 9606–9607; (b) Kovacs, H.; Kupče, Ě. *Magn. Reson. Chem.* **2016**, *54*, 544–560.
4. (a) Kupče, Ě.; Claridge, T. D. W. *Angew. Chem. Int. Ed.* **2017**, *56*, 11779–11783; (b) Kupče, Ě.; Claridge, T. D. W. *Chem. Commun.* **2018**, *54*, 7139–7142; (c) Claridge, T. D. W.; Mayzel, M.; Kupče, Ě. *Magn. Reson. Chem.* **2019**, *57*, 946–952; (d) Kupče, Ě.; Claridge, T. D. W. *J. Magn. Reson.* **2019**, *307*, 106568.

5. (a) Claridge, T. D. W., *High-Resolution NMR Techniques in Organic Chemistry*, 3rd ed.; Elsevier: Amsterdam, 2016; (b) Cavanagh, J., *Protein NMR Spectroscopy: Principles and Practice*, 2nd ed.; Academic Press: Burlington, Mass., 2007.
6. Garbow, J. R.; Weitekamp, D. P.; Pines, A. *Chem. Phys. Lett.* **1982**, *93*, 504–509.
7. (a) Palmer, A. G.; Cavanagh, J.; Wright, P. E.; Rance, M. *J. Magn. Reson.* **1991**, *93*, 151–170; (b) Kay, L.; Keifer, P.; Saarinen, T. *J. Am. Chem. Soc.* **1992**, *114*, 10663–10665; (c) Cavanagh, J.; Rance, M. *Annu. Rep. NMR Spectrosc.* **1993**, *27*, 1–58.
8. Becker, J.; Koos, M. R. M.; Schulze-Sünninghausen, D.; Luy, B. *J. Magn. Reson.* **2019**, *300*, 76–83.
9. Kupče, Ē.; Freeman, R. *Magn. Reson. Chem.* **2007**, *45*, 2–4.
10. Schulze-Sünninghausen, D.; Becker, J.; Koos, M. R. M.; Luy, B. *J. Magn. Reson.* **2017**, *281*, 151–161.
11. Koos, M. R. M.; Kummerlöwe, G.; Kaltschnee, L.; Thiele, C. M.; Luy, B. *Angew. Chem. Int. Ed.* **2016**, *55*, 7655–7659.
12. (a) Schleucher, J.; Schwendinger, M.; Sattler, M.; Schmidt, P.; Schedletzky, O.; Glaser, S. J.; Sørensen, O. W.; Griesinger, C. *J. Biomol. NMR* **1994**, *4*, 301–306; (b) Kontaxis, G.; Stonehouse, J.; Laue, E. D.; Keeler, J. *J. Magn. Reson.* **1994**, *111*, 70–76.
13. Briand, J.; Sørensen, O. W. *J. Magn. Reson.* **1997**, *125*, 202–206.
14. Pérez-Trujillo, M.; Nolis, P.; Bermel, W.; Parella, T. *Magn. Reson. Chem.* **2007**, *45*, 325–329.
15. (a) Tufts, D.; Kumaresan, R. *IEEE Trans. Acoust., Speech, Signal Process.* **1982**, *30*, 671–675; (b) Koehl, P. *Prog. Nucl. Magn. Reson. Spectrosc.* **1999**, *34*, 257–299.
16. (a) Nolis, P.; Motiram-Corral, K.; Pérez-Trujillo, M.; Parella, T. *ChemPhysChem* **2019**, *20*, 356–360; (b) Nolis, P.; Motiram-Corral, K.; Pérez-Trujillo, M.; Parella, T. *J. Magn. Reson.* **2019**, *300*, 1–7.

Supporting Information
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Diversifying NOAH Supersequences with New
HSQC-based Modules

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1 Product operator analysis for NOAH modules

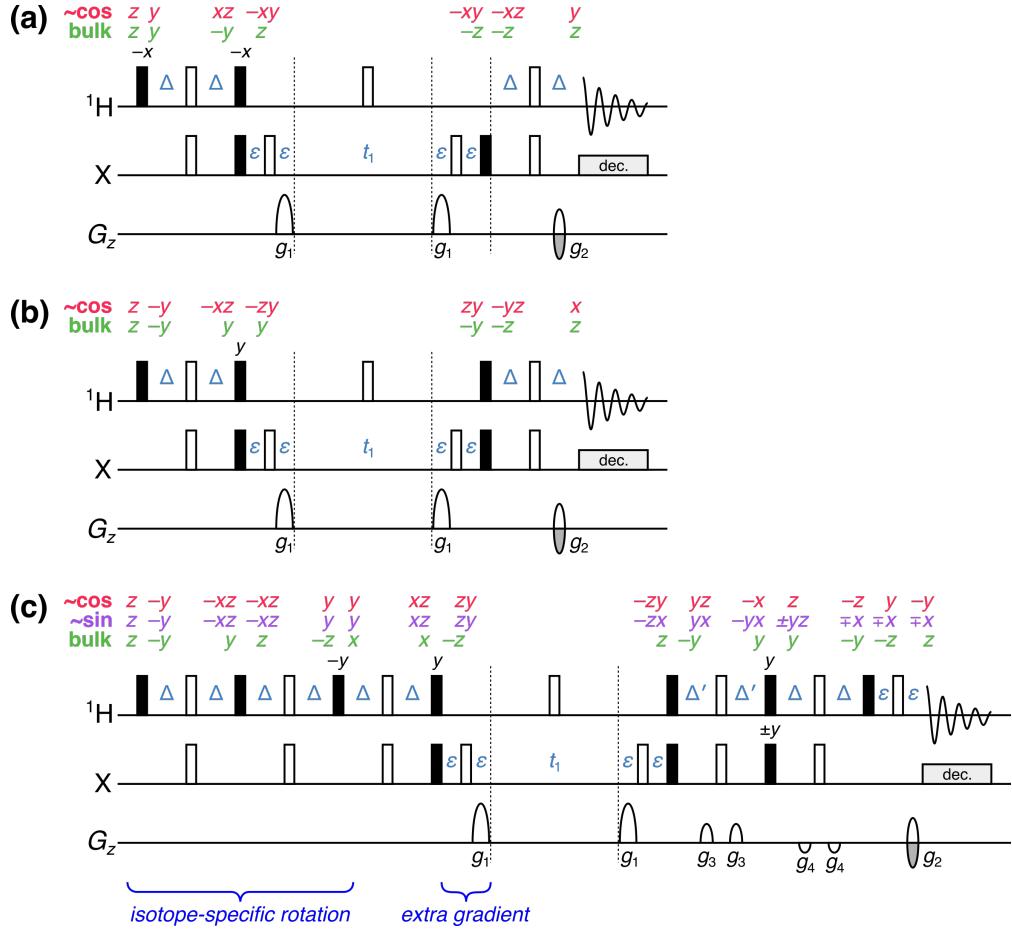


Figure S1: Product operators present at each stage of NOAH modules for an IS spin system. One-letter terms m ($m \in \{x, y, z\}$) are shorthand for single-spin terms on proton, i.e. \hat{I}_m . Two-letter terms mn are shorthand for two-spin terms on both the proton and heteronucleus, i.e. $2\hat{I}_m\hat{S}_n$. “~cos” represents the pathway for directly coupled proton magnetisation that is cosine-modulated after t_1 : for the HMQC and HSQC, this is the only component that is detected. For the seHSQC, the sine-modulated component (labelled with “~sin”) is also detected. “bulk” refers to the bulk magnetisation, i.e. protons that are not directly coupled to the heteronucleus. (a) NOAH HMQC. (b) NOAH HSQC. (c) NOAH seHSQC with ISR. Immediately following the ISR pulse sequence element, directly bonded protons are rotated onto $+y$, whereas the bulk magnetisation is rotated onto $+x$. Note that this analysis assumes $\Delta' = 1/(4 \cdot {}^1J_{\text{XH}})$.

2 Multiplicity editing in seHSQC

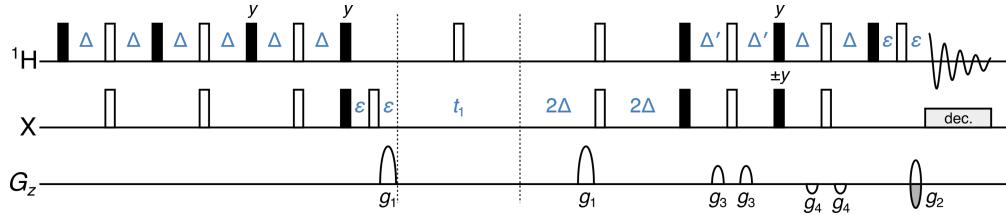


Figure S2: Implementation of multiplicity editing in the new NOAH seHSQC module. Note the different phase in the third ^1H 90° pulse ($+y$ as opposed to the $-y$ in Figure S1c). This is needed to compensate for the extra ^1H 180° pulse in the editing period. Symbols have the same meaning as in Figure 1 of the main text.

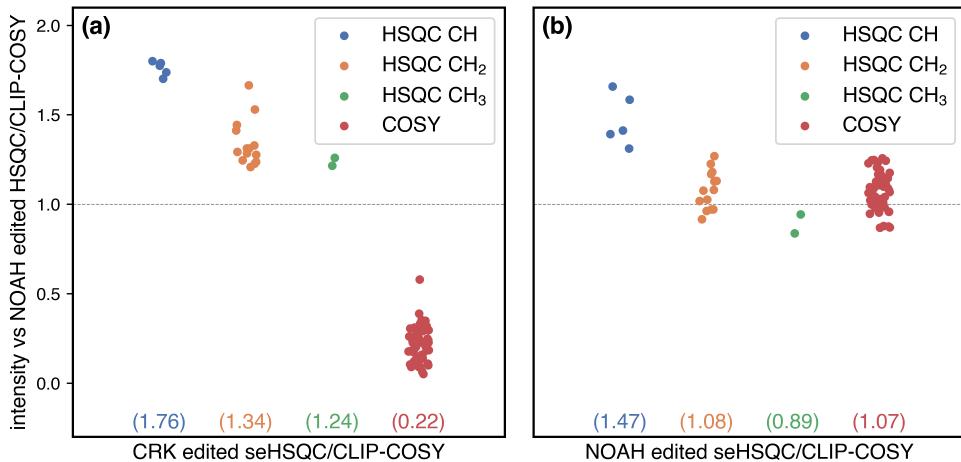


Figure S3: Sensitivity of edited seHSQC versus the NOAH HSQC/CLIP-COSY supersequence. (a) CRK edited seHSQC + CLIP-COSY. Although larger gains are observed in the HSQC, the COSY intensities are severely decreased. (b) NOAH edited seHSQC + CLIP-COSY. On average, sensitivity gains are observed in both the HSQC and COSY modules (except for HSQC CH₃ peaks). Spectra were obtained on a 700 MHz Bruker AV III equipped with a TCI H/C/N cryoprobe; the sample used was 40 mM andrographolide in DMSO-*d*₆.

3 Effect of setting $\Delta' = 1/(4 \cdot {}^1J_{\text{CH}})$ in seHSQC

The Δ' delay in the CRK and NOAH seHSQC sequences can be set to $1/(4 \cdot {}^1J_{\text{CH}})$ in order to optimise the sensitivity enhancement for CH groups only. The effects of doing so are shown here for the unedited and edited seHSQCs respectively.

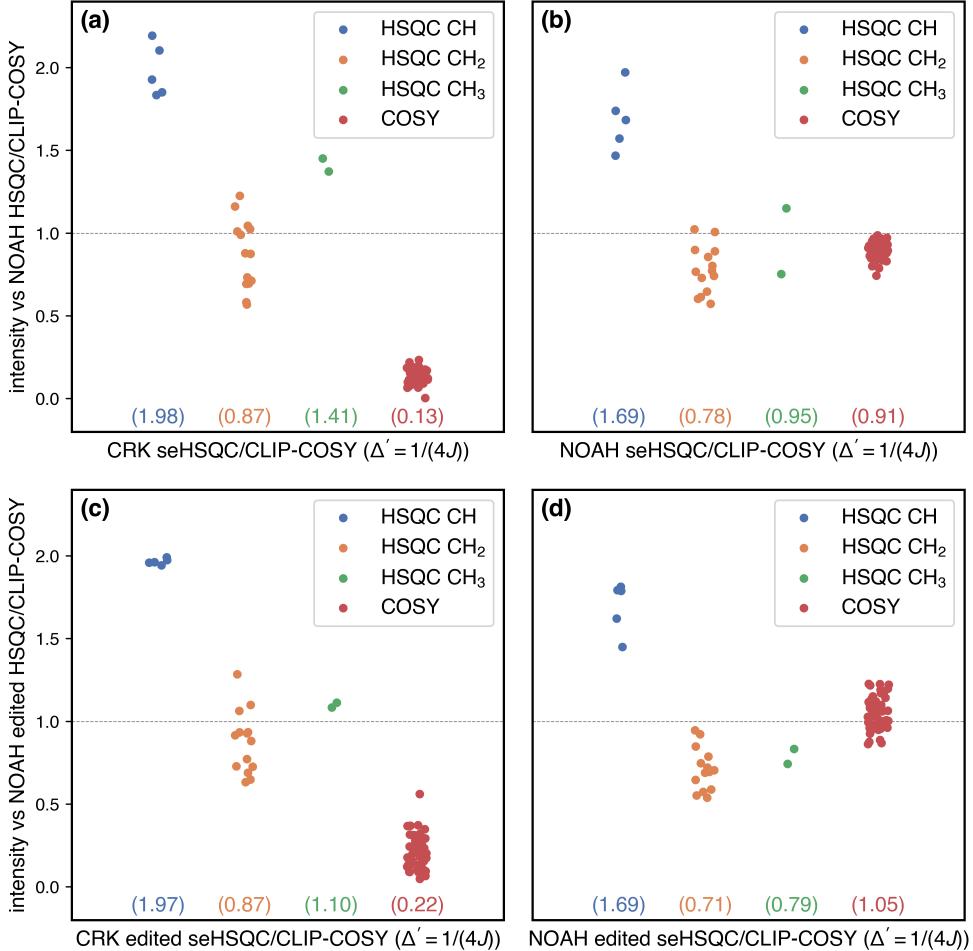


Figure S4: Sensitivity of seHSQC sequences with Δ' set to $1/(4 \cdot {}^1J_{\text{CH}})$, versus the corresponding NOAH HSQC/CLIP-COSY supersequence (i.e. unedited for (a) and (b), edited for (c) and (d)). (a) CRK seHSQC + CLIP-COSY, without multiplicity editing. (b) NOAH seHSQC + CLIP-COSY, without multiplicity editing. (c) CRK seHSQC + CLIP-COSY, with multiplicity editing. (d) NOAH seHSQC + CLIP-COSY, with multiplicity editing. Spectra were obtained on a 700 MHz Bruker AV III equipped with a TCI H/C/N cryoprobe; the sample used was 40 mM andrographolide in DMSO-*d*₆.

In particular, for the NOAH seHSQC, we note that the improvements in HSQC CH sensitivity gained by moving from $\Delta' = 1/(8 \cdot {}^1J_{\text{CH}})$ to $\Delta' = 1/(4 \cdot {}^1J_{\text{CH}})$ are marginal (ca. 10%). At the same time, sensitivity *losses* are observed for CH₂ and CH₃ peaks, likely due to pulse imperfections.

4 Comparison of BIG-BIRD and ISR elements

The BIG-BIRD element used here was $45^\circ_{45^\circ}(^1\text{H}) - 2\Delta - 180^\circ(^1\text{H}, ^{13}\text{C}) - 2\Delta - 45^\circ_{225^\circ}(^1\text{H})$ for the unedited NOAH seHSQC, where β_ϕ indicates a hard pulse with flip angle β and phase ϕ , and $\Delta = 1/(4 \cdot ^1\text{J}_{\text{CH}})$. For the edited NOAH seHSQC, the BIG-BIRD pulse phases are slightly modified to give $45^\circ_{315^\circ}(^1\text{H}) - 2\Delta - 180^\circ(^1\text{H}, ^{13}\text{C}) - 2\Delta - 45^\circ_{135^\circ}(^1\text{H})$. These, and the ISR, have the same net effect on coupled and uncoupled proton magnetisation, as shown in Figure S1. However, the ISR provides greater sensitivity in both the HSQC and downstream COSY.

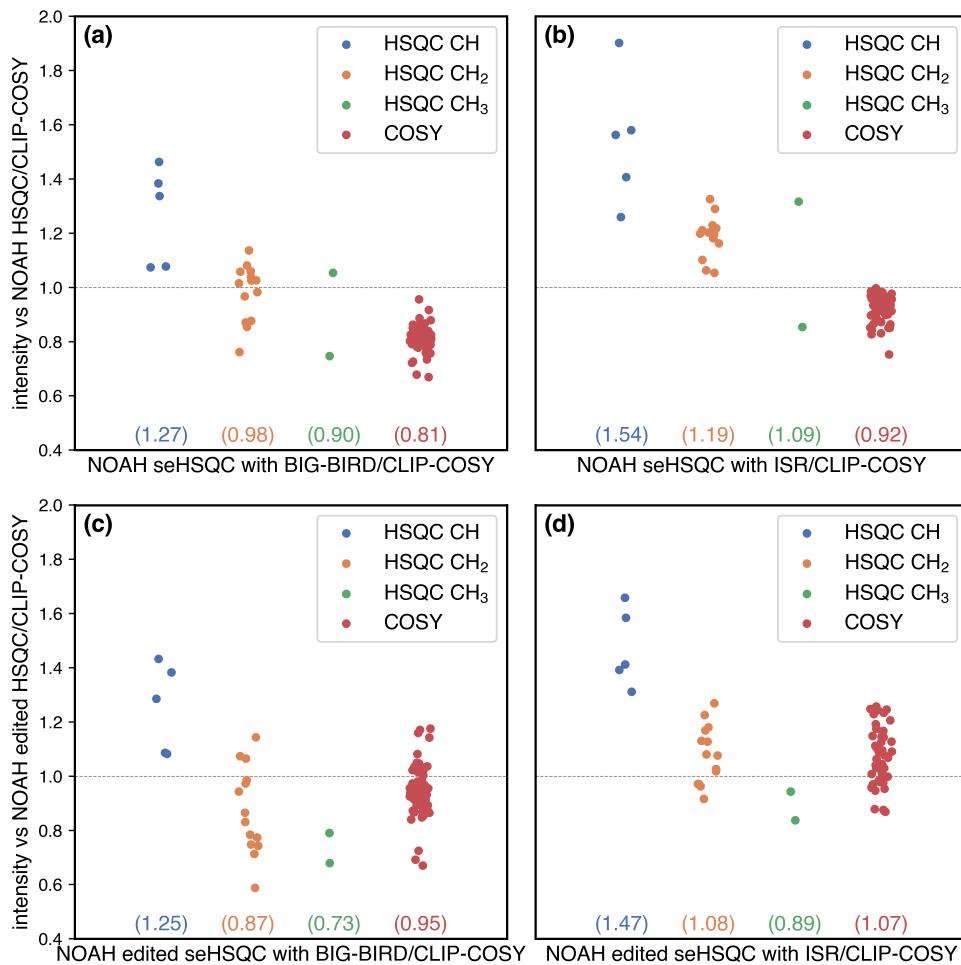


Figure S5: Sensitivity of NOAH seHSQC sequences with prepended BIG-BIRD and ISR elements, versus the corresponding NOAH HSQC/CLIP-COSY supersequence (i.e. unedited for (a) and (b), edited for (c) and (d)). (a) NOAH seHSQC with BIG-BIRD + CLIP-COSY, without multiplicity editing. (b) NOAH seHSQC with ISR + CLIP-COSY, without multiplicity editing. (c) NOAH seHSQC with BIG-BIRD + CLIP-COSY, with multiplicity editing. (d) NOAH seHSQC with ISR + CLIP-COSY, with multiplicity editing. Spectra were obtained on a 700 MHz Bruker AV III equipped with a TCI H/C/N cryoprobe; the sample used was 40 mM andrographolide in DMSO-*d*₆.

5 Suppression of wing artefacts

The origin of the “wing” artefacts in the final homonuclear modules can be most clearly seen from the following series of experiments involving the NOAH-3 ^{15}N seHSQC/ ^{13}C seHSQC/CLIP-COSY (SpnSpCc) supersequence. Since the f_1 spectral windows of the two seHSQC modules are different, they lead to distinct sets of wing artefacts if the extra gradient before t_1 is not present. Figure S6 focuses on the artefacts associated with intense methyl group peaks, but similar artefacts are observed for all other peaks, albeit with lower absolute intensities.

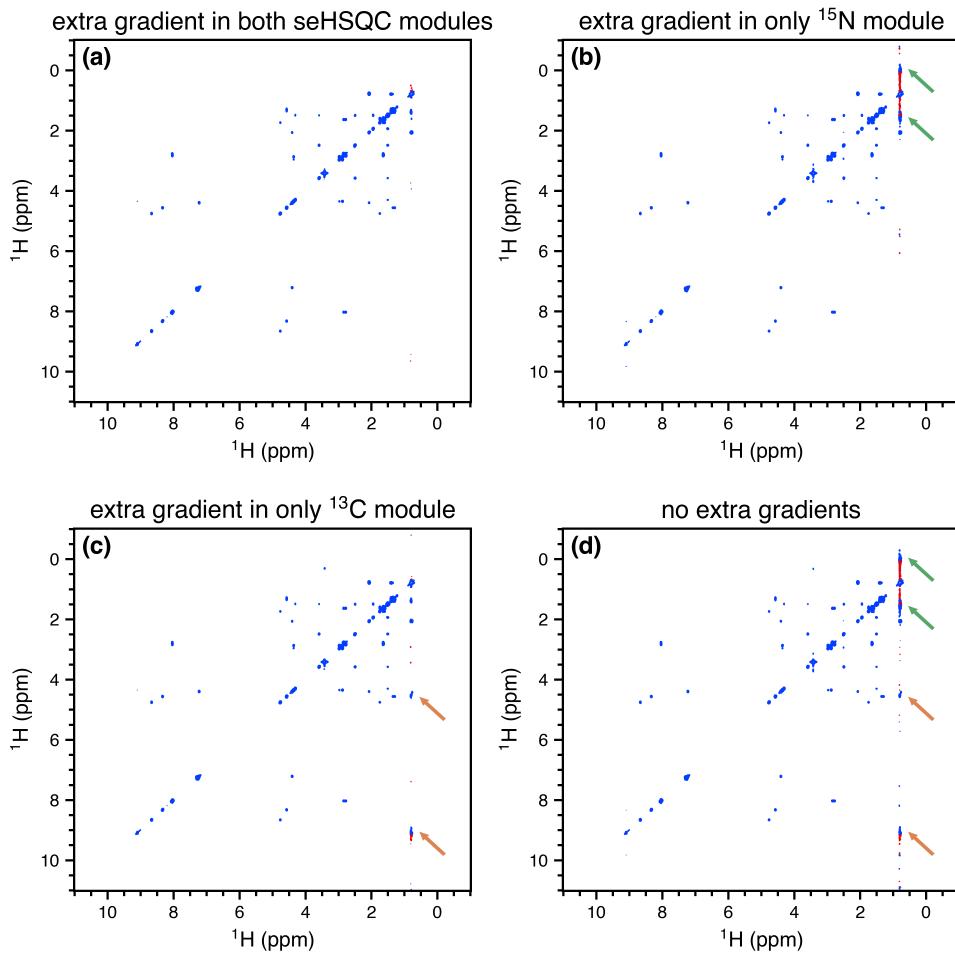


Figure S6: CLIP-COSY spectra obtained from various forms of the NOAH-3 SpnSpCc supersequence. Wing artefacts arising from the ^{15}N seHSQC are highlighted in orange; those arising from the ^{13}C seHSQC in green. Notice how (in this case) the former can easily be misinterpreted as a crosspeak, while the latter obscures genuine crosspeaks. **(a)** With the extra gradient inserted for both modules, i.e. no artefacts. **(b)** With an extra gradient in only the ^{15}N module, i.e. only the ^{13}C artefacts. **(c)** With an extra gradient in only the ^{13}C module. **(d)** With no extra gradients. Spectra were obtained on a 700 MHz Bruker AV III equipped with a TCI H/C/N cryoprobe; the sample used was 40 mM gramicidin in DMSO- d_6 .

6 ^{15}N HSQC and line broadening

For $^{15}\text{N}-^1\text{H}$ correlations, both the HMQC and the new seHSQC module are recommended as they keep the bulk magnetisation along $\pm z$ during the t_1 period. The HSQC module places bulk magnetisation in the xy -plane, leading to J_{HH} evolution; consequently, the amount of bulk magnetisation “passed on” to the downstream modules decreases as the ^{15}N t_1 is increased. Since t_1 for each NOAH module is incremented in sync, this is manifested in downstream modules as a t_1 -dependent decrease in amplitude, or f_1 line broadening after Fourier transformation, as shown in Figure S7.

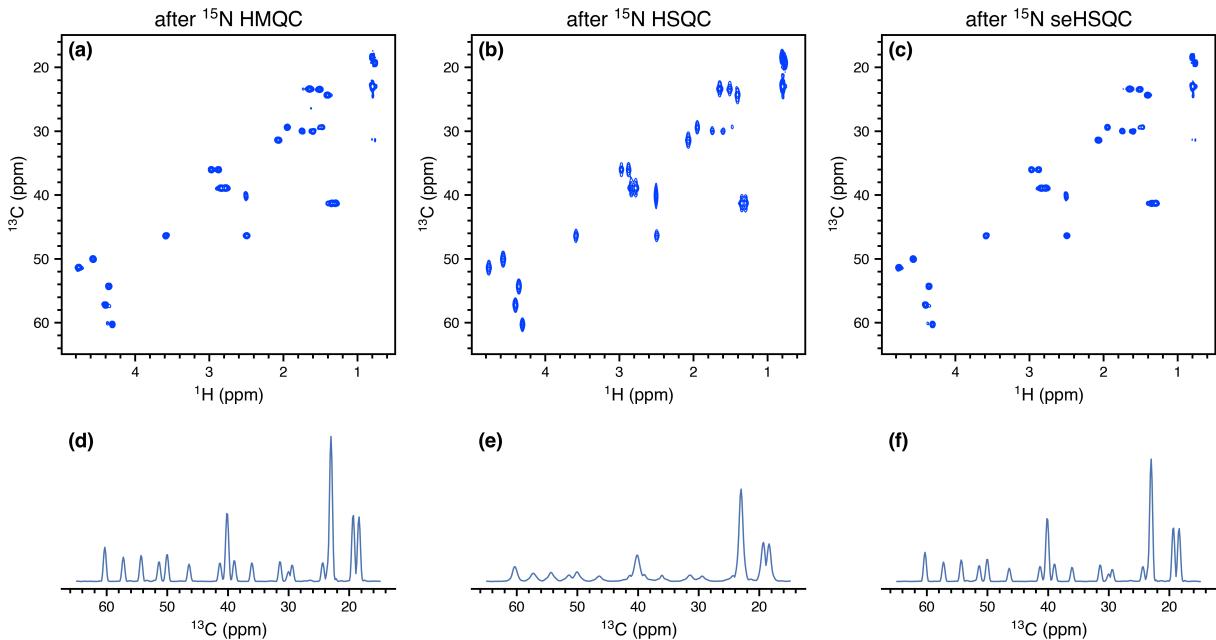


Figure S7: ^{13}C seHSQC spectra obtained from NOAH-3 XSpCc (^{15}N module + ^{13}C seHSQC + CLIP-COSY) supersequences. The ^{15}N spectral window was 30 ppm and 256 t_1 increments were collected, corresponding to an indirect-dimension ^{15}N acquisition time of 60.1 ms. (a) X = HMQC. (b) X = HSQC. (c) X = seHSQC. (d)–(f) Projections of spectra (a)–(c) onto the f_1 axis. Note the f_1 line broadening in (b) and (e). Spectra were obtained on a 700 MHz Bruker AV III equipped with a TCI H/C/N cryoprobe; the sample used was 40 mM gramicidin in $\text{DMSO}-d_6$.

This line broadening also leads to a substantial sensitivity loss (almost 65% in Figure S7). The extent of the line broadening depends on the acquisition time, and is particularly pronounced for long acquisition times, i.e. small ^{15}N spectral windows. In our experience, at ^{15}N acquisition times of ca. 5 ms the effect is almost indiscernible. Such a short acquisition time would lead to poor resolution in the ^{15}N dimension itself, which may or may not be tolerable. Of course, this issue can be entirely avoided by using either the HMQC or seHSQC.

7 Effect of lengthened gradients in ^{15}N experiments

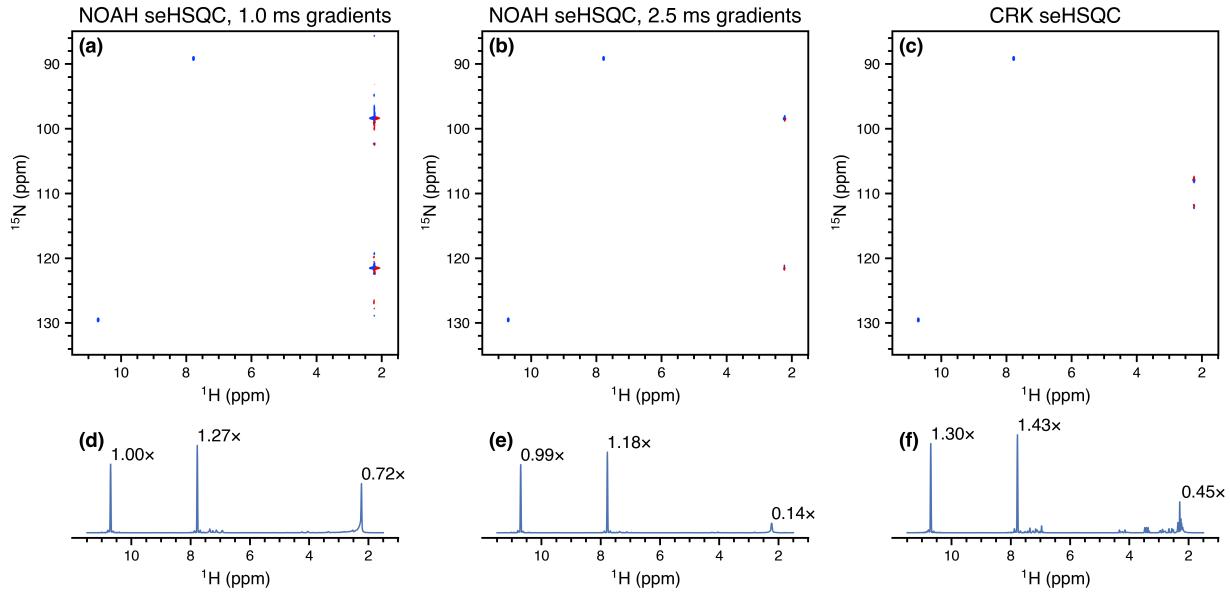


Figure S8: ^{15}N seHSQC spectra obtained using the NOAH and CRK implementations. The peaks at 7.8 and 10.7 ppm (^1H shifts) are genuine crosspeaks; the mixed-phase peaks at 2.2 ppm are artefacts. (a) NOAH seHSQC, with original CTP gradients of 1 ms. (b) NOAH seHSQC, with longer CTP gradients of 1 ms. (c) Standalone CRK seHSQC with 1 ms CTP gradients. (d)–(f) Projections of spectra (a)–(c) onto the f_2 axis. The numbers indicate relative peak heights (normalised against the 10.7 ppm peak in (d)). Spectra were obtained on a 700 MHz Bruker AV III equipped with a TCI H/C/N cryoprobe; the sample used was 50 mM zolmitriptan in $\text{DMSO}-d_6$.

The lengthening of CTP gradients from 1 ms to 2.5 ms is aimed at cleaning up artefacts arising from bulk magnetisation that is not properly returned to $+z$ at the end of the sequence. Figure S8 shows exactly how effective this strategy is. In (d), where the CTP gradients have their original duration, the artefacts have comparable intensity to the desired peaks. When the gradients are lengthened in (e), the crosspeak intensities are almost unaffected (with losses of < 10% arising perhaps from relaxation and diffusion). However, the artefacts are suppressed by a factor of 5 or more. Although this suppression is not complete, this should not be interpreted as a weakness of the new NOAH seHSQC module, as similar artefacts are also visible in the CRK seHSQC (f). Indeed, every $^{15}\text{N}-^1\text{H}$ experiment we tested has at least *some* artefact intensity in this region.

8 Effect of k -scaling

The effect of k -scaling on the HMQC is shown in Figure S9. By decreasing the indirect dimension resolution, the f_1 linewidths of the peaks increase: this can lead to significant sensitivity enhancement for the HMQC (up to $2.7\times$), because J_{HH} splitting in the f_1 dimension is no longer resolved. The largest gains are observed for peaks where J_{HH} splitting is more visible; for the leftmost peak at $\Omega_N = 128$ ppm, only $1.7\times$ gains in sensitivity can be attained through this method.

For the seHSQC module, k -scaling on its own leads to far smaller sensitivity gains (Figure S10). Any increase in the total peak volume is almost completely offset by the f_1 broadening. Therefore, even at $k = 8$, the largest sensitivity gains that can be attained are $\sim 1.3\times$.

The use of linear prediction for spectra with $k > 1$ can, to a certain extent, compensate for the line broadening. This is less successful for the HMQC spectra (Figure S11). Although raw gains in peak height can be observed for all values of k , there is a corresponding decrease in the spectral quality, as evidenced by the f_1 multiplet structure being increasingly distorted. On the other hand, linear prediction performs well for the seHSQC spectra (Figure S12), where there is no multiplet structure in f_1 . Even the reconstruction with $k = 8$ has reasonable spectral quality: although the 2D spectrum (d) appears to have unusual peak shapes, this is merely the result of having the same contour levels as the $k = 1$ spectrum. The actual peaks are still clearly singlets, as can be seen from the projection in (h).

An additional example of successful k -scaling and linear prediction (with $k = 4$) can be seen in Section 10.

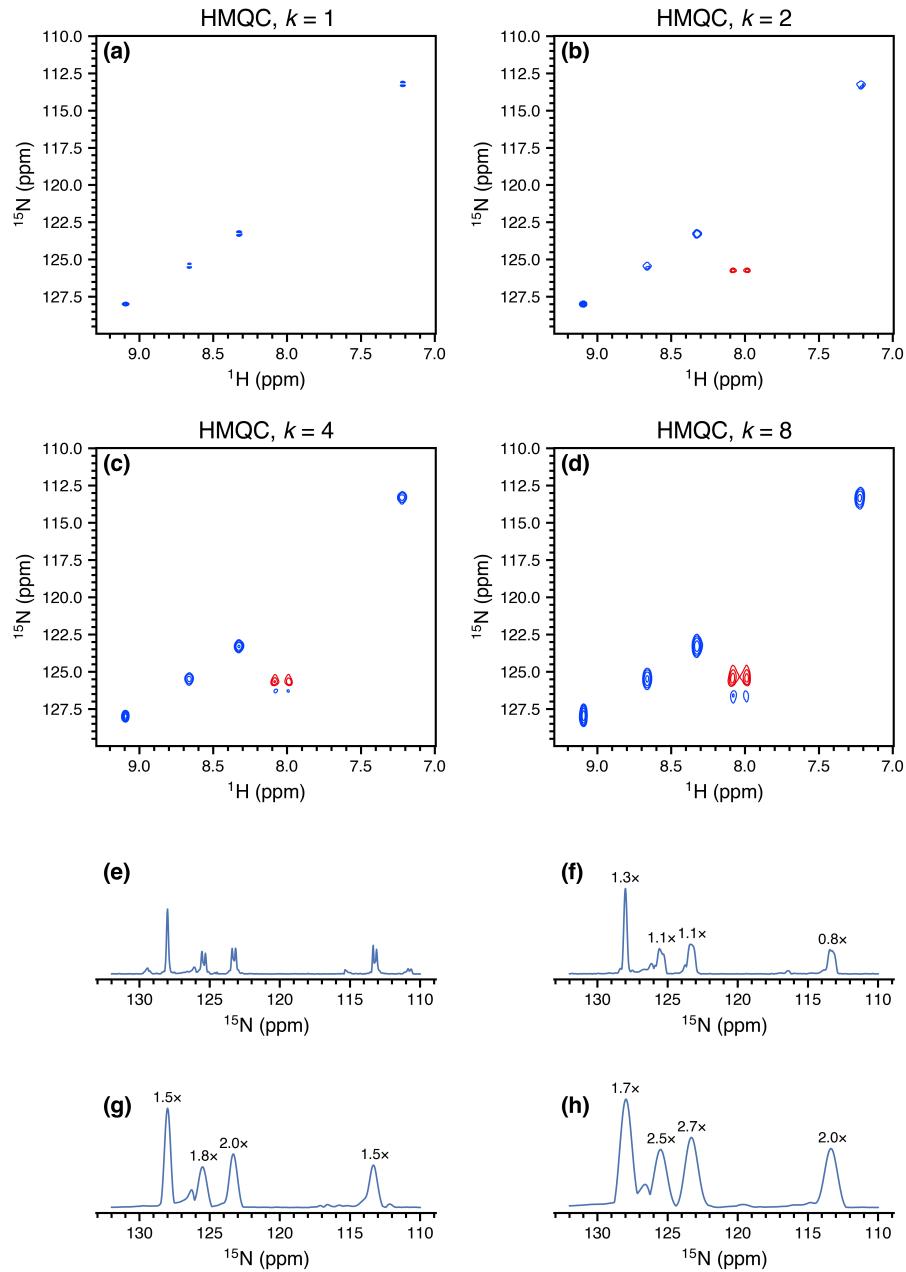


Figure S9: (HMQC without linear prediction.) ¹⁵N HMQC spectra (from NOAH-3 MSpCc supersequences) obtained with various values of the scaling factor k . The peak at $\Omega_H = 8.03$ ppm is a folded peak from the ornithine δ -NH₂. (a) $k = 1$, with 256 t_1 increments and 2 scans per increment. (b) $k = 2$, i.e. effectively 128 t_1 increments and 4 scans per increment. (c) $k = 4$. (d) $k = 8$. (e)–(h) Projections of 2D spectra in (a)–(d) onto the f_1 axis. Numbers indicate peak intensities relative to the $k = 1$ HMQC spectrum. Spectra were obtained on a 700 MHz Bruker AV III equipped with a TCI H/C/N cryoprobe; the sample used was 40 mM gramicidin in DMSO-*d*₆.

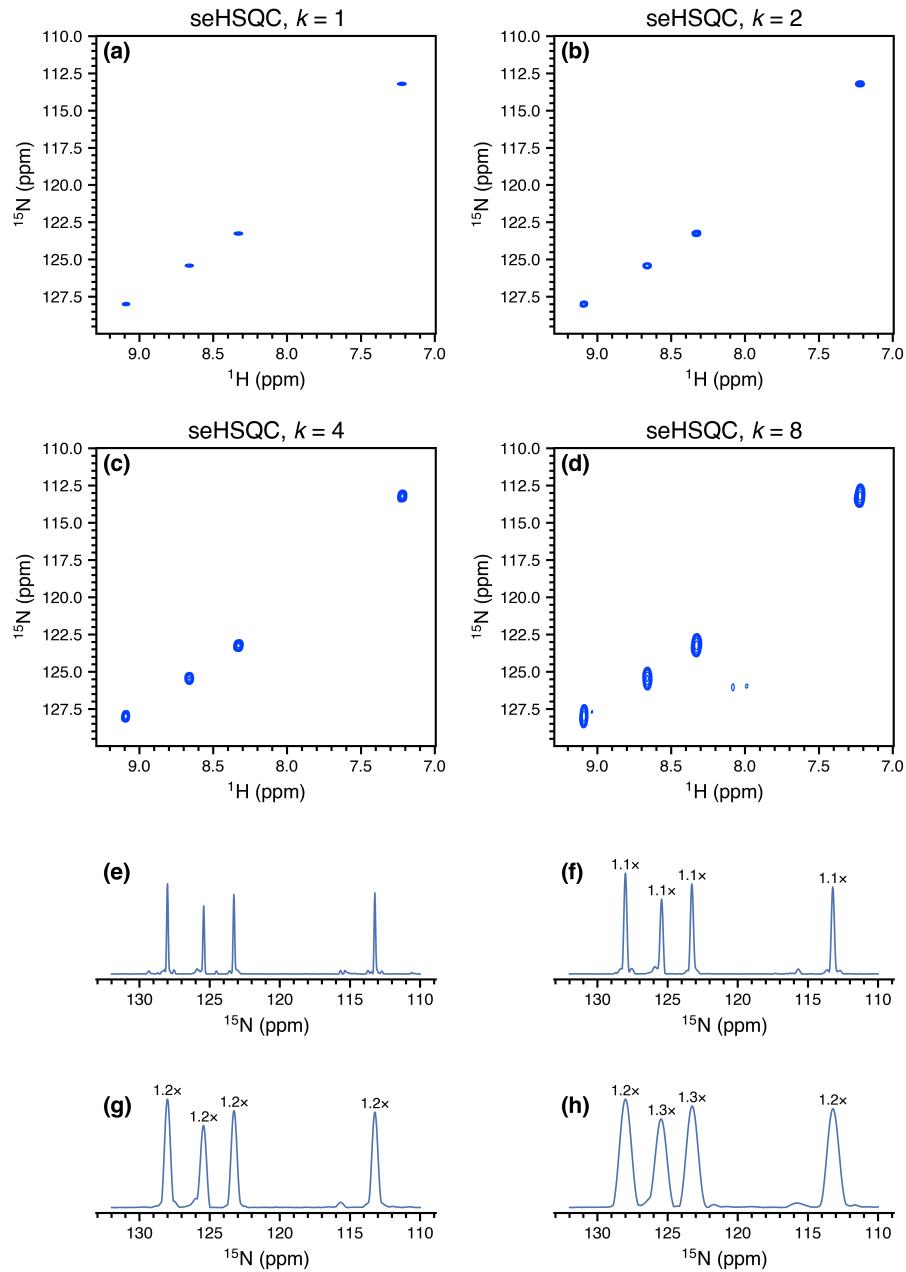


Figure S10: (seHSQC without linear prediction.) ¹⁵N seHSQC spectra (from NOAH-3 SpnSpCc supersequences) obtained with various values of the scaling factor k . The peak at $\Omega_H = 8.03$ ppm is a folded peak from the ornithine $\delta\text{-NH}_2$. (a) $k = 1$, with 256 t_1 increments and 2 scans per increment. (b) $k = 2$, i.e. effectively 128 t_1 increments and 4 scans per increment. (c) $k = 4$. (d) $k = 8$. (e)–(h) Projections of 2D spectra in (a)–(d) onto the f_1 axis. Numbers indicate peak intensities relative to the $k = 1$ seHSQC spectrum. Spectra were obtained on a 700 MHz Bruker AV III equipped with a TCI H/C/N cryoprobe; the sample used was 40 mM gramicidin in DMSO-*d*₆.

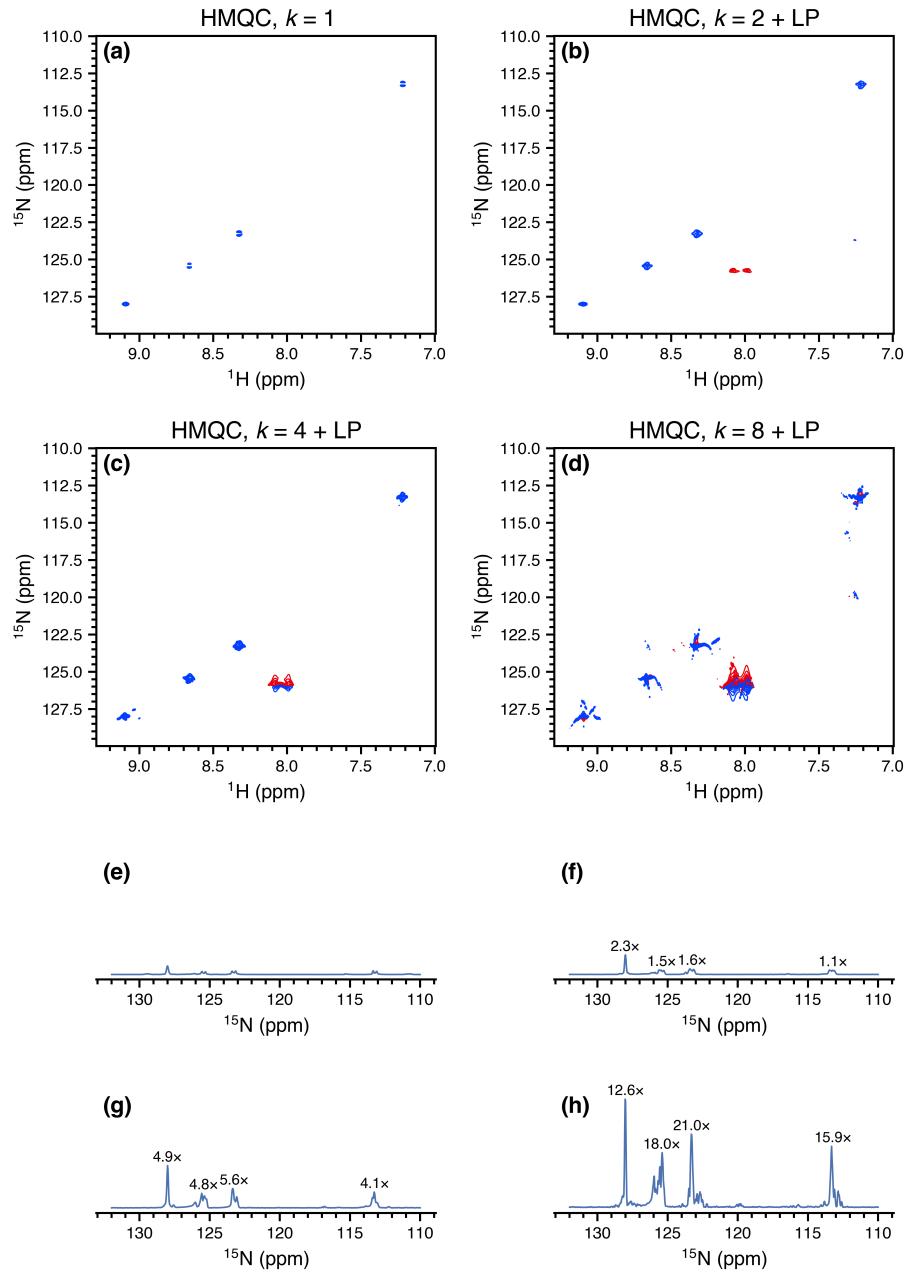


Figure S11: (HMQC with linear prediction.) ^{15}N HMQC spectra (from NOAH-3 MSpCc supersequences) obtained with various values of the scaling factor k , after linear prediction up to 512 complex points in f_1 . The peak at $\Omega_{\text{H}} = 8.03$ ppm is a folded peak from the ornithine $\delta\text{-NH}_2$. (a) $k = 1$. Note that this spectrum is the same as in Figure S9a. (b) $k = 2$. (c) $k = 4$. (d) $k = 8$. (e)–(h) Projections of 2D spectra in (a)–(d) onto the f_1 axis. Numbers indicate peak intensities relative to the $k = 1$ HMQC spectrum. Spectra were obtained on a 700 MHz Bruker AV III equipped with a TCI H/C/N cryoprobe; the sample used was 40 mM gramicidin in DMSO- d_6 .

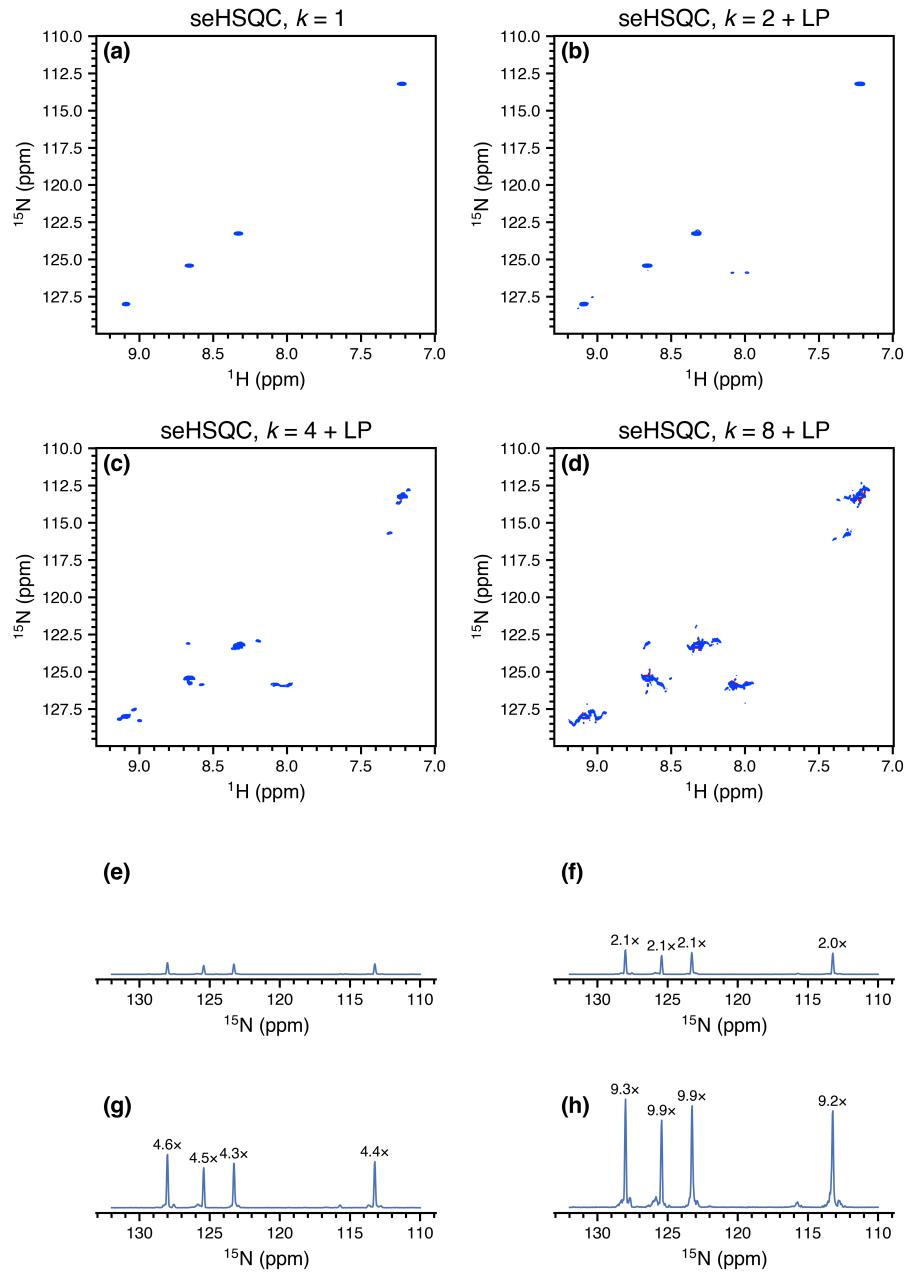


Figure S12: (seHSQC with linear prediction.) ^{15}N seHSQC spectra (from NOAH-3 SpnSpCc super-sequences) obtained with various values of the scaling factor k , after linear prediction up to 512 complex points in f_1 . The peak at $\Omega_{\text{H}} = 8.03$ ppm is a folded peak from the ornithine $\delta\text{-NH}_2$. (a) $k = 1$. Note that this spectrum is the same as in Figure S9a. (b) $k = 2$. (c) $k = 4$. (d) $k = 8$. (e)–(h) Projections of 2D spectra in (a)–(d) onto the f_1 axis. Numbers indicate peak intensities relative to the $k = 1$ seHSQC spectrum. Spectra were obtained on a 700 MHz Bruker AV III equipped with a TCI H/C/N cryoprobe; the sample used was 40 mM gramicidin in DMSO- d_6 .

9 HSQC-TOCSY/HSQC sensitivity comparisons

The signal intensities for the NOAH-3 StSCc (HSQC-TOCSY + HSQC + CLIP-COSY) supersequences can be more conveniently measured by omitting the DIPSI-2 isotropic mixing in the HSQC-TOCSY supersequence, leading to a NOAH-3 SSCc (HSQC + HSQC + CLIP-COSY) supersequence. This allows us to compare the different versions of double-HSQC sequences, as the two HSQC modules can be implemented either using the MFA approach, or the new ASAP/NOAH approach based on Ernst angle excitation in the first module. In the latter implementation, the parameter f can be varied between 0.4 and 1; it represents the proportion of $^{13}\text{C}-\text{H}$ magnetisation used in the first HSQC, as described in the main text. Furthermore, to boost the sensitivity of the second HSQC module in the NOAH supersequences, the new seHSQC module can be used in place of it.

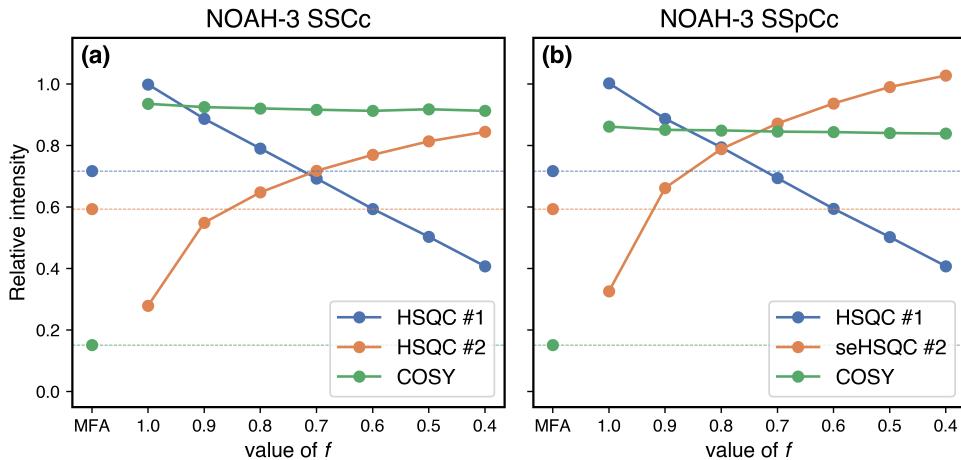


Figure S13: Sensitivities of HSQC and CLIP-COSY modules when used as part of a SSCc-type supersequence, with both the NOAH and MFA implementations of the two HSQC modules. Intensities are calculated relative to the HSQC and CLIP-COSY modules in a standard NOAH-2 SCc supersequence (averaged over all peaks). The MFA SSCc sensitivities are on the left of each plot; horizontal dashed lines at these levels are drawn to guide the eye. (a) Sensitivity of NOAH-3 SSCc modules as a function of f . (b) Sensitivity of NOAH-3 SSpcC modules (the second HSQC module is replaced with seHSQC) as a function of f . Spectra were obtained on a 700 MHz Bruker AV III equipped with a TCI H/C/N cryoprobe; the sample used was 40 mM andrographolide in DMSO- d_6 .

Figure S13 can be understood in the following way:

- The MFA HSQC sensitivities (on the left of both plots) are approximately half that of a standard CRK seHSQC, with the second HSQC having slightly lower sensitivity. This is discussed in ref. 16 of the main text.
- The sensitivity of the first NOAH HSQC (blue) is generally equal to f , supporting the interpretation of f as the fraction of $^{13}\text{C}-\text{H}$ magnetisation excited in the first HSQC.

- The sensitivity of the second NOAH HSQC (orange) arises from whatever is *not* used by the first HSQC, plus any magnetisation that relaxes during the FID of the first HSQC. As f is decreased, the former contribution increases and the latter tapers off. This is true for the seHSQC as well (in Figure S13b), except that there is a uniform boost in sensitivity for all values of f . This sensitivity improvement mainly applies to CH groups, as discussed in the main text.
- The MFA COSY sensitivity is substantially lower ($\sim 15\%$) because the bulk magnetisation is dephased by the previous modules, whereas in the NOAH approach it is (largely) preserved.

It remains to evaluate the impact of adding DIPSI-2 mixing in one of the HSQC modules on the remaining modules in the supersequence. This depends on whether the HSQC-TOCSY module is placed first (StSC or StSpC) or second (SStC) in the sequence. Because of the possibility of using the seHSQC module in the other slot, we generally recommend placing the HSQC-TOCSY sequence first. As can be seen **TODO**

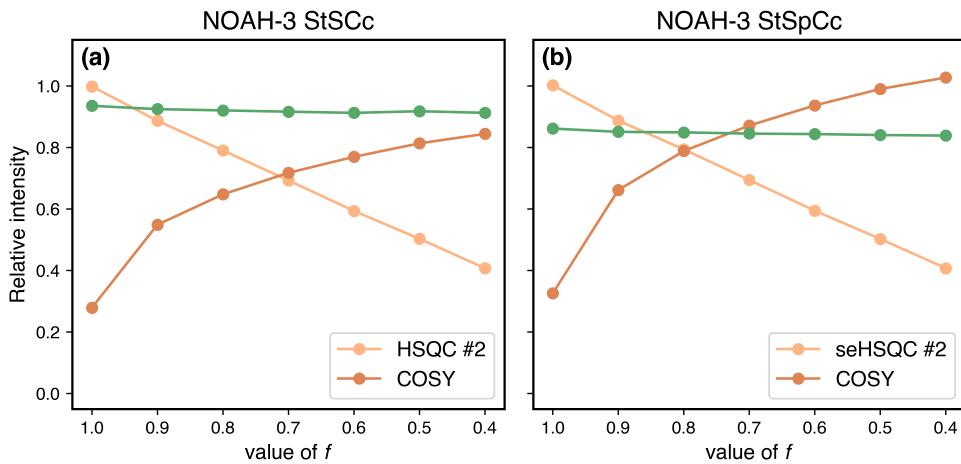


Figure S14: Blah. Spectra were obtained on a 700 MHz Bruker AV III equipped with a TCI H/C/N cryoprobe; the sample used was 40 mM andrographolide in DMSO-*d*₆.

10 Other example spectra

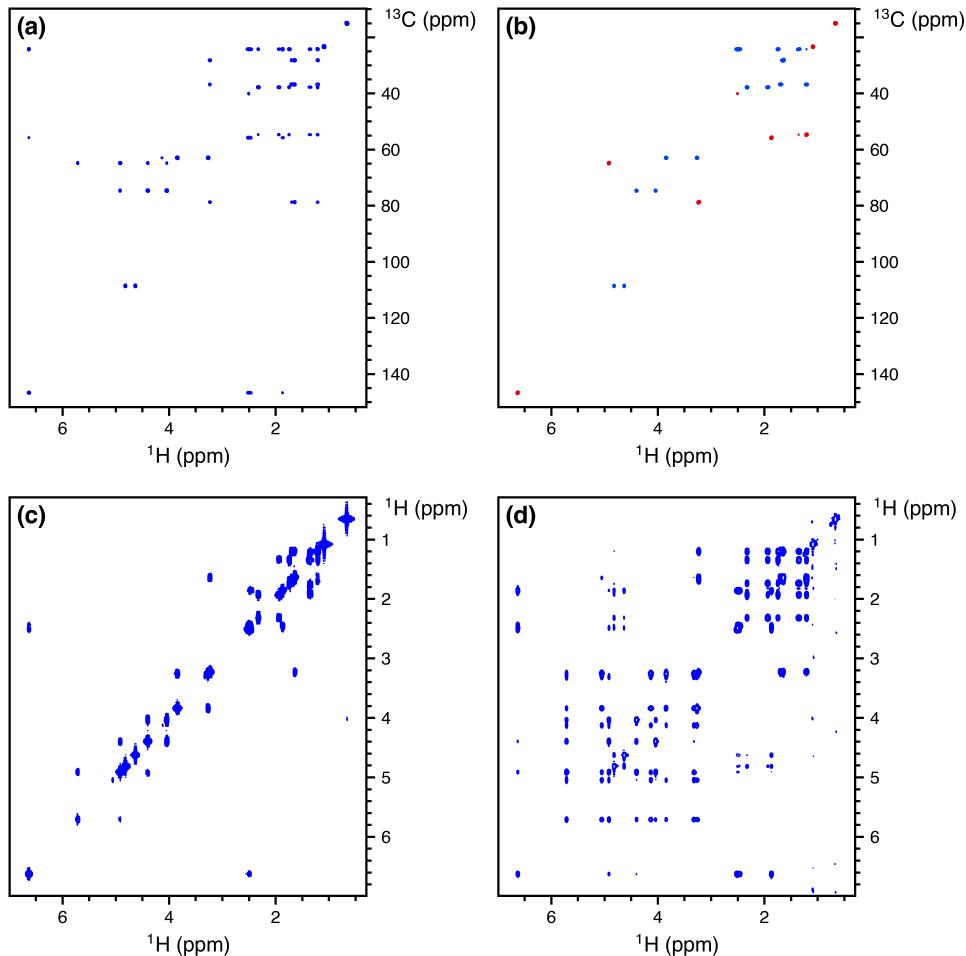


Figure S15: 2D spectra acquired using the NOAH-4 StSpCT supersequence. 256 t_1 increments were used with 2 scans per increment, leading to a total experiment time of 17 minutes and 32 seconds. This represents a 3.25 \times time saving relative to conventional acquisition of each of the four spectra with the same parameters, which would take a total of 57 minutes and 3 seconds. (a) HSQC-TOCSY (30 ms mixing time, $f = 0.9$). (b) Multiplicity edited seHSQC. (c) COSY. (d) TOCSY (60 ms mixing time). Spectra were obtained on a 700 MHz Bruker AV III equipped with a TCI H/C/N cryoprobe; the sample used was 40 mM andrographolide in DMSO- d_6 .

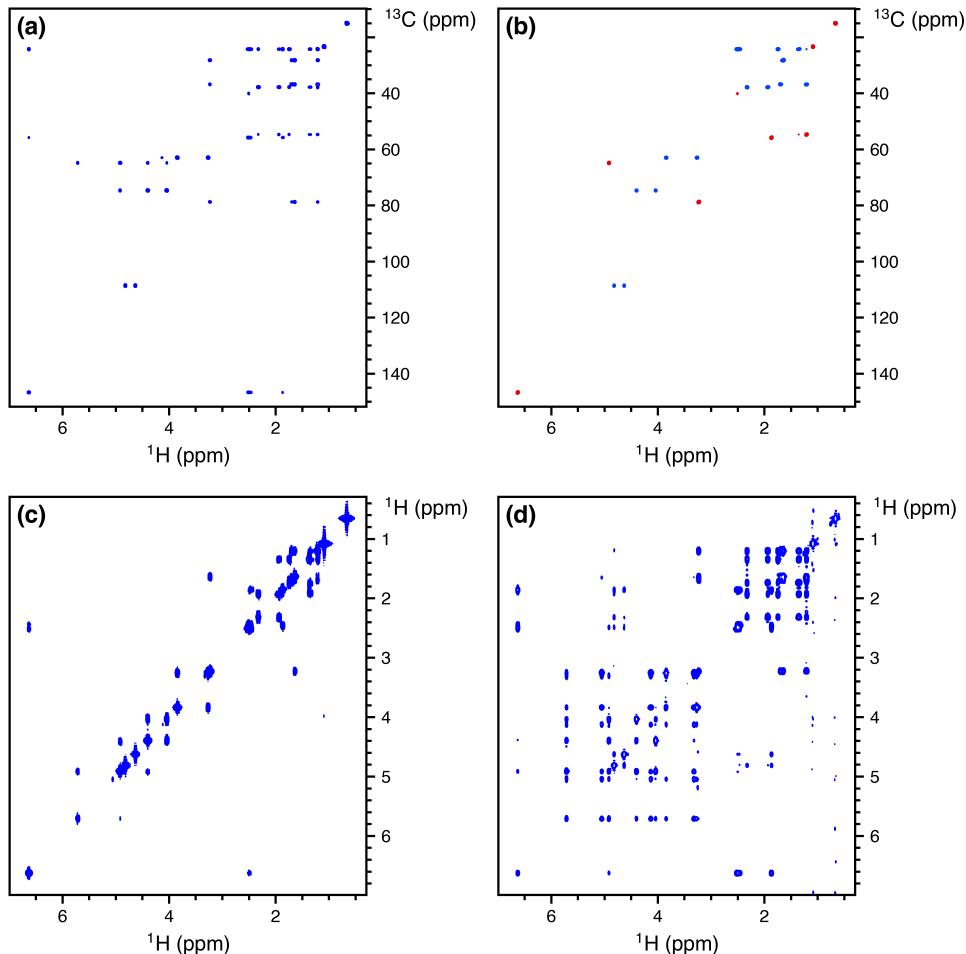


Figure S16: 2D spectra acquired using the NOAH-4 StSpCT supersequence with 50% non-uniform sampling. All other parameters are the same as in Figure S15. The experimental time was 9 minutes and 1 second. (a) HSQC-TOCSY (30 ms mixing time, $f = 0.9$). (b) Multiplicity edited seHSQC. (c) COSY. (d) TOCSY (60 ms mixing time). Spectra were obtained on a 700 MHz Bruker AV III equipped with a TCI H/C/N cryoprobe; the sample used was 40 mM andrographolide in $\text{DMSO}-d_6$.

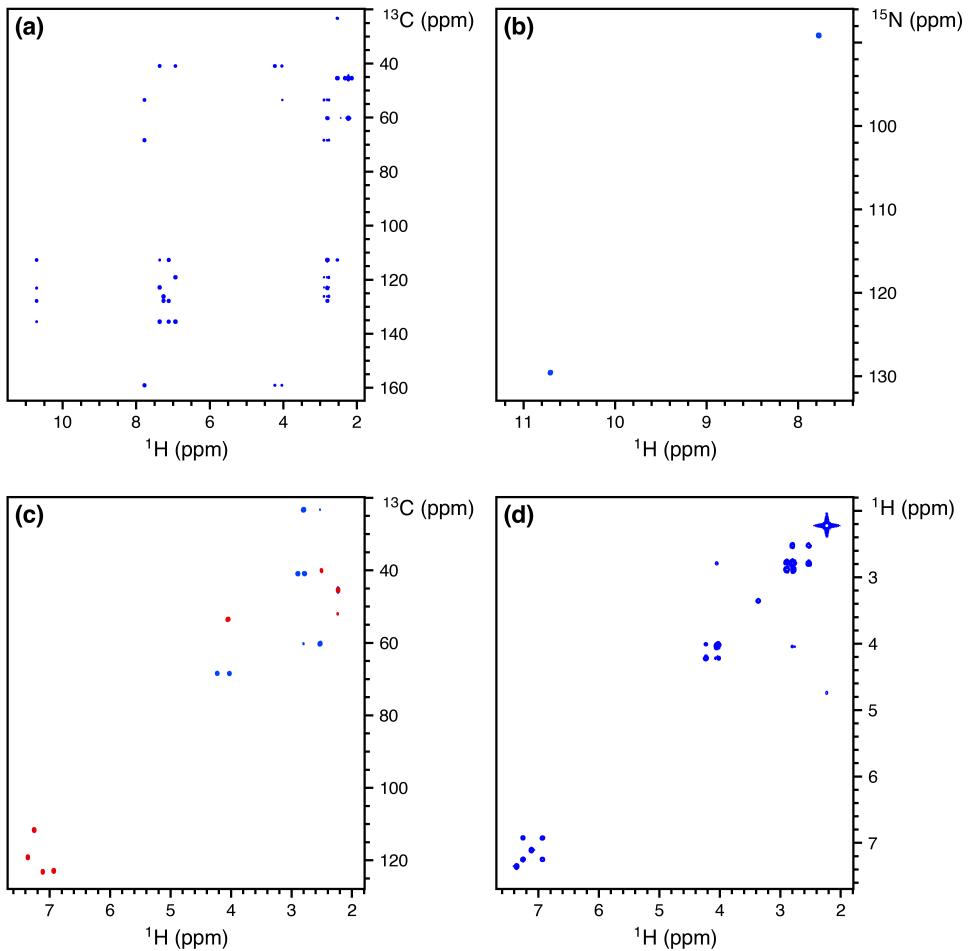


Figure S17: 2D spectra acquired using the NOAH-4 BSpnSpCqf supersequence. 256 t_1 increments were used with 2 scans per increment, leading to a total experiment time of 17 minutes and 32 seconds. This represents a 3.22 \times time saving relative to conventional acquisition of each of the four spectra with the same parameters, which would take a total of 56 minutes and 28 seconds. (a) HMBC. (b) ^{15}N seHSQC with $k = 4$ and linear projected to 512 complex points. (c) Multiplicity edited ^{13}C seHSQC. (d) Magnitude-mode COSY. Spectra were obtained on a 700 MHz Bruker AV III equipped with a TCI H/C/N cryoprobe; the sample used was 50 mM zolmitriptan in DMSO- d_6 .

11 Pulse programmes

I have not fleshed out this part and the next. Firstly, it makes the SI really really long (StSpCc is 10 pages long!!), and for not much purpose anyway: I am of the opinion that users are better served by downloading a file rather than copying pages of text from a PDF – which can go quite wrong sometimes as odd characters and page numbers get copied too! Secondly, it is quite easy to do this up, as L^AT_EX provides mechanisms by which the entire contents of a file can be included. So it can be done at a later stage.

Alternatively, we can point people to Bruker library, or nmrweb.chem, or (depending on which journal we go to) they might allow zip files as a supplementary file.

11.1 NOAH-2 SpCc: seHSQC + CLIP-COSY

```
; noah2-SpCc

; 13C sensitivity-enhanced HSQC
;      [use -DEDIT for multiplicity editing]
; 1H CLIP-COSY (States)

;CLASS=HighRes
;DIM=2D
;TYPE=
;SUBTYPE=
;COMMENT=

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

#ifndef NUS
define list<loopcounter> t1list=<$VCLIST>
#endif

define delay DC_SEHSQC1
define delay DC_SEHSQC2
define delay DC_SEHSQC3
define delay DC_SEHSQC4
define delay DC_SEHSQC5
define delay DC_SEHSQC6
define delay DC_SEHSQC7
define delay DC_SEHSQC8
define delay DC_SEHSQC9
define delay DC_SEHSQC10
"cnst41 = 2*sfo2/sfo1"           ; gradient ratio
"d0      = 3u"                     ; 13C HSQC t1
"d2      = 0.5s/cnst2"             ; JCOMP
"d4      = 0.25s/cnst2"            ; 13C INEPT
```

```

"d10      = 3u"                      ; CLIP-COSY t1
"d12      = 0.25s/cnst12"            ; CLIP-COSY mixing (< 1/4J(HH))
"in0      = inf1/2"                  ; 13C HSQC increment
"in10     = 2*dw"                   ; CLIP-COSY increment
"p2       = p1*2"                   ; 1H hard 180
"DC_SEHSQC1 = d4-p14/2"             ; zz-filter
"DC_SEHSQC2 = d4+p14/2"             ; zz-filter
"DC_SEHSQC3 = d4-larger(p2,p14)/2" ; INEPT
"DC_SEHSQC4 = p16+d16+p2+d0*2-4u-p3*2/PI" ; 13C pre-t1 if editing
"DC_SEHSQC5 = d2-p16-d16+p3*2/PI"    ; 13C editing period
"DC_SEHSQC6 = d2-p2-p3*2/PI"        ; 13C editing period
"DC_SEHSQC7 = p16+d16+p2/2+d0-4u-p3*2/PI" ; 13C pre-/post-t1 if no editing
"DC_SEHSQC8 = d6-cnst17*p24/2-p19-d16" ; first spin echo after t1
"DC_SEHSQC9 = d4-larger(p2,p14)/2-p16-d16" ; second spin echo after t1
"DC_SEHSQC10= p16+d16-p1*0.78+de+8u"   ; final spin echo for refocusing gradient
define list<gradient> GC_SEHSQC={cnst41}
"l0      = td1/4"                   ; Total number of 13C t1 increments
"l1      = 0"                       ; Running counter of 13C t1 increments
"l2      = 0"                       ; Counter, even for echo, odd for antiecho
"acqt0   = 0"
baseopt_echo

1 ze
2 30m
3 5m
4 50u UNBLKGRAD

#define NUS
  "d0=(in0*t1list)+3u"
  "d10=(in10*t1list)+3u"
#endif

; Cleanup
4u p12:f2
(p3 ph0):f2
4u p11:f1
p16:gp0
d16
(p1 ph0):f1
4u
p16:gp0*-1.37
d16
(p1 ph1):f1
4u
p16:gp0*0.77
d16
4u BLKGRAD
d1 st0
4u UNBLKGRAD

```

```

; MODULE 1
; 13C-1H seHSQC version 2

; reverse zz-filter
(p1 ph0):f1
DC_SEHSQC1
(p14:sp3 ph0):f2
(p2 ph0):f1
DC_SEHSQC2
(p1 ph0):f1
DC_SEHSQC1
(p14:sp3 ph0):f2
(p2 ph0):f1
DC_SEHSQC2           ; 13C-1H: y, 12C-1H: z

; forward INEPT
#ifndef EDIT
  (p1 ph1):f1
#else
  (p1 ph3):f1
#endif
DC_SEHSQC3
4u
(center (p2 ph0):f1 (p14:sp3 ph0):f2 )
4u
DC_SEHSQC3 p12:f2
4u
(p1 ph1):f1 (p3 ph5):f2

; t1 evolution with optional multiplicity editing
#ifndef EDIT
  4u
  DC_SEHSQC4
  (p31:sp18 ph0):f2
  p16:gp4
  d16 p12:f2

  d0
  (p2 ph7):f1
  d0

  p16:gp4
  d16
  DC_SEHSQC5
  (p31:sp18 ph0):f2
  (p2 ph0):f1
  DC_SEHSQC6 p12:f2
#else
  4u
  DC_SEHSQC7
  (p24:sp7 ph0):f2

```

```

p16:gp4
d16 p12:f2

d0
(p2 ph7):f1
d0

p16:gp4
d16
(p24:sp7 ph0):f2
4u
DC_SEHSQC7 p12:f2
#endif

; reverse INEPT for first component
(center (p1 ph0):f1 (p3 ph7):f2 )
p19:gp6
d16
DC_SEHSQC8
(center (p2 ph0):f1 (p24:sp7 ph0):f2 )
DC_SEHSQC8
p19:gp6
d16 p12:f2
(center (p1 ph1):f1 (p3 ph9):f2 ) ; seHSQC pulse, incremented with EA

; reverse INEPT for second component
p16:gp7
d16
DC_SEHSQC9
(center (p2 ph0):f1 (p14:sp3 ph0):f2 )
DC_SEHSQC9
p16:gp7
d16
(p1 ph0):f1

; spin echo for refocusing gradient
DC_SEHSQC10
(p2 ph0):f1
4u
p16:gp4*EA*GC_SEHSQC
d16 p12:f2
4u
goscnp ph30 cpd2:f2 ; acquire 13C HSQC
50u do:f2

; Cleanup
4u p11:f1
4u p12:f2
(p3 ph0):f2
4u
p16:gp0*1.77

```

```

2m st

; MODULE 2
; 1H-1H CLIP-COSY

(p1 ph6):f1
d10
(p1 ph0):f1
10u gron12 p10:f1
(p32:sp29 ph0):f1
20u groff
d16 p11:f1
(p1 ph0):f1

; in-phase transfer via perfect echo
d12
(p2 ph1):f1
d12
(p1 ph1):f1
d12
(p2 ph3):f1
d12

(p1 ph0):f1
10u gron12*1.333 p10:f1
(p32*0.75:sp29 ph2):f1
20u groff
p16:gp11
d16 p11:f1
4u
(p1 ph0):f1

go=2 ph26

; echo/antiecho loop
"l2 = l2 + 1"
1m igrad EA
1m ip6
1m ip9*2
30m wr #0 if #0 zd
lo to 3 times 2

; t1 incrementation
"l1 = l1 + 1"
#endif NUS
1m t1list.inc
#endif
1m ip5*2
1m rp6
1m ip30*2

```

```

; 13C t1 incrementation
#ifndef NUS
#else
  1m id0
#endif

; 1H t1 incrementation
#ifndef NUS
#else
  1m id10
#endif /* NUS */

lo to 4 times 10

50u BLKGRAD
exit

ph0=0
ph1=1
ph2=2
ph3=3
ph5=0 2
ph6=0 2
ph7=0 0 2 2
ph9=1 1 3 3
ph26=0 2
ph30=0 2 2 0

;gpnam0: SMSQ10.100
;gpnam4: SMSQ10.100
;gpnam6: SMSQ10.100
;gpnam7: SMSQ10.100
;gpnam11: SMSQ10.100
;gpz0: 24% (for purging)
;gpz4: 70% (13C CTP)
;gpz6: 11% (13C HSQC spin echo CTP)
;gpz7: -5% (13C HSQC spin echo CTP)
;gpz11: 43% (1H purge gradient)
;gpz12: 11% (1H ZQ filter)

;WaveMaker shaped pulses (use 'wvm -a' to generate)
;sp3:wvm:wu180C13: cawurst-20(60 kHz, 0.5 ms; L2H)
;sp18:wvm:wu180Jcomp: cawurst-40(280 ppm; Jcomp, L2H)
;cpd2:wvm:wudec: cawurst_d-20(220 ppm, 1.4 ms; L2H)

;cnst2: = 1J(CH)
;cnst12: > J(HH), ca. 30 Hz for CLIP-COSY mixing
;cnst17: = -0.5 for Crp60comp.4
;cnst41: gradient ratio (2*C/H)
;cpd2: 13C decoupling according to sequence defined by cpdprg2

```

```

;d0: 13C t1
;d1: relaxation delay
;d2: 1/2J(CH)
;d4: 1/4J(CH)
;d6: 1/8J(CH) for all multiplicities, 1/4J(CH) for CH only
;d10: 1H t1
;d12: <1/4J(HH) CLIP-COSY mixing time
;d16: delay for homospoil/gradient recovery [200 us]
;l0: total number of t1 increments
;l1: running counter of t1 increments
;l2: even for echo, odd for antiecho
;p1: f1 channel - 90 degree high power pulse
;p2: f1 channel - 180 degree high power pulse
;p3: f2 channel - 90 degree high power pulse
;p14: f2 channel - 180 degree shaped pulse for inversion
;      = 500usec for Crp60,0.5,20.1
;p16: gradient pulse [1 ms]
;p19: gradient pulse 2 [600 us]
;p24: f2 channel - 180 degree shaped pulse for refocusing
;      = 2msec for Crp60comp.4
;p31: f2 channel - 180 degree shaped pulse for inversion with J-compensation
;p32: f1 channel - 180 degree shaped pulse (adiabatic) [20 msec]
;      smoothed chirp (sweepwidth, 20% smoothing, 10000 points)
;p10: 0 W
;p11: f1 channel - power level for pulse (default)
;p12: f2 channel - power level for pulse (default)
;p112: f2 channel - power level for CPD/BB decoupling
;sp3: f2 channel - shaped pulse (180 degree inversion)
;sp7: f2 channel - shaped pulse (180 degree refocusing)
;sp18: f2 channel - shaped pulse (180 degree with J-compensation)
;sp29: f1 channel - shaped pulse (adiabatic)
;spnam3: Crp60,0.5,20.1 or WaveMaker
;spnam7: Crp60comp.4
;spnam18: Crp60_xfilt.2 or WaveMaker
;spnam29: Crp60,20,20.10
;aq: acquisition time
;ds: >= 16
;FnMODE: echo-antiecho
;NBL: number of blocks (NOAH modules)
;ns: 1 * n

; auprog: noah_hsqc:noah_clipcosy States
; ngn-2.0.0: constructed from C_SEHSQC, H_CLIP_COSY

```

11.2 NOAH-3 StSpCc: HSQC-TOCSY + seHSQC + CLIP-COSY

```

; noah3-StSpCc

; 13C HSQC-TOCSY with Ernst angle excitation
;      [specify fraction of 1J(CH) magnetisation to use with cnst32]
; 13C sensitivity-enhanced HSQC

```

```

;      [use -DEDIT for multiplicity editing]
; 1H CLIP-COSY (States)

;$CLASS=HighRes
;$DIM=2D
;$TYPE=
;$SUBTYPE=
;$COMMENT=

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

#ifndef NUS
define list<loopcounter> t1list=<$VCLIST>
#endif

define delay DC_SEHSQC1
define delay DC_SEHSQC2
define delay DC_SEHSQC3
define delay DC_SEHSQC4
define delay DC_SEHSQC5
define delay DC_SEHSQC6
define delay DC_SEHSQC7
define delay DC_SEHSQC8
define delay DC_SEHSQC9
define delay DC_SEHSQC10
define delay DHSQCT_INEPT1
define delay DHSQCT_INEPT2
define delay DHSQCT_INEPT3
define delay DHSQCT_INEPT4
define delay DHSQCT_INEPT5
define delay DHSQCT_INEPT6
"cnst41 = 2*sfo2/sfo1"           ; gradient ratio
"d0     = 3u"                     ; 13C t1
"d2     = 0.5s/cnst2"             ; JCOMP
"d4     = 0.25s/cnst2"            ; 13C INEPT
"d10    = 3u"                     ; CLIP-COSY t1
"d12    = 0.25s/cnst12"          ; CLIP-COSY mixing (< 1/4J(HH))
"in0   = inf1/2"                 ; 13C increment
"in10  = 2*dw"                   ; CLIP-COSY increment
"l13   = (d19/(p6*115.112))/2"  ; half the number of HSQC-TOCSY DIPSI-2 loops
"l14   = l13*2"                  ; number of HSQC-TOCSY DIPSI-2 loops
"p2    = p1*2"                   ; 1H hard 180
"DC_SEHSQC1 = d4-p14/2"          ; zz-filter
"DC_SEHSQC2 = d4+p14/2"          ; zz-filter
"DC_SEHSQC3 = d4-larger(p2,p14)/2" ; INEPT
"DC_SEHSQC4 = p16+d16+p2+d0*2-4u-p3*2/PI" ; 13C pre-t1 if editing
"DC_SEHSQC5 = d2-p16-d16+p3*2/PI"   ; 13C editing period
"DC_SEHSQC6 = d2-p2-p3*2/PI"       ; 13C editing period
"DC_SEHSQC7 = p16+d16+p2/2+d0-4u-p3*2/PI" ; 13C pre-/post-t1 if no editing

```

```

"DC_SEHSQC8 = d6-cnst17*p24/2-p19-d16"      ; first spin echo after t1
"DC_SEHSQC9 = d4-larger(p2,p14)/2-p16-d16"  ; second spin echo after t1
"DC_SEHSQC10= p16+d16-p1*0.78+de+8u"        ; final spin echo for refocusing gradient
"DHSQCT_INEPT1   = (asin(cnst32)/(2*PI*cnst2))-p14/2000000"
"DHSQCT_INEPT2   = (asin(cnst32)/(2*PI*cnst2))+p14/2000000"
"DHSQCT_INEPT3   = p16+d16+p2/2+d0-p3*2/PI+4u"
"DHSQCT_INEPT4   = d4-p14/2"
"DHSQCT_INEPT5   = d4+p14/2-p16-d16"
"DHSQCT_INEPT6   = de+4u"
define list<gradient> GC_SEHSQC={cnst41}
define list<gradient> GHSQCT_INEPT={cnst41}
"10      = td1/6"                      ; Total number of 13C t1 increments
"11      = 0"                          ; Running counter of 13C t1 increments
"12      = 0"                          ; Counter, even for echo, odd for antiecho
"acqt0  = 0"
baseopt_echo

1 ze
2 30m
3 5m
4 50u UNBLKGRAD

#endif NUS
"do=(in0*t1list)+3u"
"di0=(in10*t1list)+3u"
#endif

; Cleanup
4u p12:f2
(p3 ph0):f2
4u p11:f1
p16:gp0
d16
(p1 ph0):f1
4u
p16:gp0*-1.37
d16
(p1 ph1):f1
4u
p16:gp0*0.77
d16
4u BLKGRAD
d1 st0
4u UNBLKGRAD

; MODULE 1
; 13C-1H HSQC-TOCSY with Ernst angle excitation

; INEPT
(p1 ph0):f1

```

```

DHSQCT_INEPT1
(p14:sp3 ph0):f2
(p2 ph0):f1
DHSQCT_INEPT2 p12:f2
(p1 ph1):f1
(p3 ph5):f2
DHSQCT_INEPT3

; t1 period
(p14:sp3 ph0):f2
4u
p16:gp3
d16
d0
(p2 ph11):f1
d0
4u
p16:gp3
d16
(p14:sp3 ph0):f2
DHSQCT_INEPT3 p12:f2

; reverse INEPT
(p3 ph7):f2
(p1 ph2):f1
DHSQCT_INEPT4
(p14:sp3 ph0):f2
(p2 ph1):f1

p16:gp13
d16
DHSQCT_INEPT5 p110:f1

;begin DIPSI2
5 p6*3.556 ph3
p6*4.556 ph1
p6*3.222 ph3
p6*3.167 ph1
p6*0.333 ph3
p6*2.722 ph1
p6*4.167 ph3
p6*2.944 ph1
p6*4.111 ph3

p6*3.556 ph1
p6*4.556 ph3
p6*3.222 ph1
p6*3.167 ph3
p6*0.333 ph1
p6*2.722 ph3
p6*4.167 ph1

```

```

p6*2.944 ph3
p6*4.111 ph1

p6*3.556 ph1
p6*4.556 ph3
p6*3.222 ph1
p6*3.167 ph3
p6*0.333 ph1
p6*2.722 ph3
p6*4.167 ph1
p6*2.944 ph3
p6*4.111 ph1

p6*3.556 ph3
p6*4.556 ph1
p6*3.222 ph3
p6*3.167 ph1
p6*0.333 ph3
p6*2.722 ph1
p6*4.167 ph3
p6*2.944 ph1
p6*4.111 ph3
    lo to 5 times 114
;end DIPSI2

4u
p16:gp13*-1
d16 p11:f1

DHSQCT_INEPT6
(p2 ph1):f1
4u
p16:gp3*GHSQCT_INEPT*EA
d16 p12:f2
4u
goscnp ph30 cpd2:f2 ; acquire 13C HSQC-TOCSY
50u do:f2

; Cleanup
4u p11:f1
4u p12:f2
(p3 ph0):f2
4u
p16:gp0*1.77
2m st

; MODULE 2
; 13C-1H seHSQC version 2

; reverse zz-filter

```

```

(p1 ph0):f1
DC_SEHSQC1
(p14:sp3 ph0):f2
(p2 ph0):f1
DC_SEHSQC2
(p1 ph0):f1
DC_SEHSQC1
(p14:sp3 ph0):f2
(p2 ph0):f1
DC_SEHSQC2 ; 13C-1H: y, 12C-1H: z

; forward INEPT
#ifndef EDIT
(p1 ph1):f1
#else
(p1 ph3):f1
#endif
DC_SEHSQC3
4u
(center (p2 ph0):f1 (p14:sp3 ph0):f2 )
4u
DC_SEHSQC3 p12:f2
4u
(p1 ph1):f1 (p3 ph5):f2

; t1 evolution with optional multiplicity editing
#ifndef EDIT
4u
DC_SEHSQC4
(p31:sp18 ph0):f2
p16:gp4
d16 p12:f2

d0
(p2 ph7):f1
d0

p16:gp4
d16
DC_SEHSQC5
(p31:sp18 ph0):f2
(p2 ph0):f1
DC_SEHSQC6 p12:f2
#else
4u
DC_SEHSQC7
(p24:sp7 ph0):f2
p16:gp4
d16 p12:f2

d0

```

```

(p2 ph7):f1
d0

p16:gp4
d16
(p24:sp7 ph0):f2
4u
DC_SEHSQC7 p12:f2
#endif

; reverse INEPT for first component
(center (p1 ph0):f1 (p3 ph7):f2 )
p19:gp6
d16
DC_SEHSQC8
(center (p2 ph0):f1 (p24:sp7 ph0):f2 )
DC_SEHSQC8
p19:gp6
d16 p12:f2
(center (p1 ph1):f1 (p3 ph9):f2 ) ; seHSQC pulse, incremented with EA

; reverse INEPT for second component
p16:gp7
d16
DC_SEHSQC9
(center (p2 ph0):f1 (p14:sp3 ph0):f2 )
DC_SEHSQC9
p16:gp7
d16
(p1 ph0):f1

; spin echo for refocusing gradient
DC_SEHSQC10
(p2 ph0):f1
4u
p16:gp4*EA*GC_SEHSQC
d16 p12:f2
4u
goscnp ph30 cpd2:f2 ; acquire 13C HSQC
50u do:f2

; Cleanup
4u p11:f1
4u p12:f2
(p3 ph0):f2
4u
p16:gp0*2.32
2m st

; MODULE 3

```

```

; 1H-1H CLIP-COSY

(p1 ph6):f1
d10
(p1 ph0):f1
10u gron12 p10:f1
(p32:sp29 ph0):f1
20u groff
d16 p11:f1
(p1 ph0):f1

; in-phase transfer via perfect echo
d12
(p2 ph1):f1
d12
(p1 ph1):f1
d12
(p2 ph3):f1
d12

(p1 ph0):f1
10u gron12*1.333 p10:f1
(p32*0.75:sp29 ph2):f1
20u groff
p16:gp11
d16 p11:f1
4u
(p1 ph0):f1

go=2 ph26

; echo/antiecho loop
"l2 = l2 + 1"
1m igrad EA
1m ip6
1m ip9*2
30m wr #0 if #0 zd
lo to 3 times 2

; t1 incrementation
"l1 = l1 + 1"
#endif NUS
1m t1list.inc
#endif
1m ip5*2
1m rp6
1m ip30*2

; 13C t1 incrementation
#endif NUS
#else

```

```

1m id0
#endif

; 1H t1 incrementation
#ifndef NUS
#else
1m id10
#endif /* NUS */

lo to 4 times 10

50u BLKGRAD
exit

ph0=0
ph1=1
ph2=2
ph3=3
ph5=0 2
ph6=0 2
ph7=0 0 2 2
ph9=1 1 3 3
ph11=0 0 0 2 2 2 2
ph26=0 2
ph30=0 2 2 0

;gpnam0: SMSQ10.100
;gpnam3: SMSQ10.100
;gpnam4: SMSQ10.100
;gpnam6: SMSQ10.100
;gpnam7: SMSQ10.100
;gpnam11: SMSQ10.100
;gpnam13: SMSQ10.100
;gpz0: 24% (for purging)
;gpz3: 75% (HSQC-TOCSY CTP)
;gpz4: 70% (13C CTP)
;gpz6: 11% (13C HSQC spin echo CTP)
;gpz7: -5% (13C HSQC spin echo CTP)
;gpz11: 43% (1H purge gradient)
;gpz12: 11% (1H ZQ filter)
;gpz13: 19% (HSQC-TOCSY purge gradients)

;WaveMaker shaped pulses (use 'wvm -a' to generate)
;sp3:wvm:wu180C13: cawurst-20(60 kHz, 0.5 ms; L2H)
;sp18:wvm:wu180Jcomp: cawurst-40(280 ppm; Jcomp, L2H)
;cpd2:wvm:wudec: cawurst_d-20(220 ppm, 1.4 ms; L2H)

;cnst2: = 1J(CH)
;cnst12: > J(HH), ca. 30 Hz for CLIP-COSY mixing
;cnst17: = -0.5 for Crp60comp.4
;cnst32: fraction of C-H magn used for HSQC-TOCSY [0.5 to 1]

```

```

;cnst41: gradient ratio (2*C/H)
;cpd2: 13C decoupling according to sequence defined by cpdprg2
;d0: 13C t1
;d1: relaxation delay
;d2: 1/2J(CH)
;d4: 1/4J(CH)
;d6: 1/8J(CH) for all multiplicities, 1/4J(CH) for CH only
;d10: 1H t1
;d12: <1/4J(HH) CLIP-COSY mixing time
;d16: delay for homospoil/gradient recovery [200 us]
;d19: HSQC-TOCSY mixing time
;l0: total number of t1 increments
;l1: running counter of t1 increments
;l2: even for echo, odd for antiecho
;l13: HSQC-TOCSY: half the number of DIPSI-2 cycles
;l14: HSQC-TOCSY: actual number of DIPSI-2 cycles
;p1: f1 channel - 90 degree high power pulse
;p2: f1 channel - 180 degree high power pulse
;p3: f2 channel - 90 degree high power pulse
;p6: f1 channel - 90 degree low power pulse
;p14: f2 channel - 180 degree shaped pulse for inversion
;      = 500usec for Crp60,0.5,20.1
;p16: gradient pulse [1 ms]
;p19: gradient pulse 2 [600 us]
;p24: f2 channel - 180 degree shaped pulse for refocusing
;      = 2msec for Crp60comp.4
;p31: f2 channel - 180 degree shaped pulse for inversion with J-compensation
;p32: f1 channel - 180 degree shaped pulse (adiabatic) [20 msec]
;      smoothed chirp (sweepwidth, 20% smoothing, 10000 points)
;p10: 0 W
;p11: f1 channel - power level for pulse (default)
;p12: f2 channel - power level for pulse (default)
;p110: f1 channel - power level for TOCSY-spinlock
;p112: f2 channel - power level for CPD/BB decoupling
;sp3: f2 channel - shaped pulse (180 degree inversion)
;sp7: f2 channel - shaped pulse (180 degree refocusing)
;sp18: f2 channel - shaped pulse (180 degree with J-compensation)
;sp29: f1 channel - shaped pulse (adiabatic)
;spnam3: Crp60,0.5,20.1 or WaveMaker
;spnam7: Crp60comp.4
;spnam18: Crp60_xfilt.2 or WaveMaker
;spnam29: Crp60,20,20.10
;aq: acquisition time
;ds: >= 16
;FnMODE: echo-antiecho
;NBL: number of blocks (NOAH modules)
;ns: 1 * n

; auprog: noah_hsqc:noah_hsqc:noah_clipcosy States
; ngn-2.0.0: constructed from HSQCT_INEPT, C_SEHSQC, H_CLIP_COSY

```

12 Processing scripts

...