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NOAH – NMR Supersequences for Small Molecule Analysis and Structure Elucidation

Ēriks Kupče[b] and Tim Claridge*[a]

Abstract: Nested NMR experiments combining up to five conventional NMR pulse sequences into one supersequence are introduced. The core two-dimensional NMR techniques routinely employed in small molecule NMR, such as HSQC, HMQC, HMBC, COSY, NOESY, TOCSY and similar, can be recorded in a single measurement dramatically reducing the data collection time and increasing sample throughput for basic NMR applications to structure elucidation and verification in synthetic, medicinal and natural product chemistry.

The structure characterization of small molecules by NMR spectroscopy nowadays largely follows well established protocols that are reliant on a core set of 2D correlation experiments that includes COSY, TOCSY, NOESY/ROESY, HSQC, HMQC, and HMBC sequences, or variants of these.[1] Having established themselves as the primary techniques, much focus has now turned to developing experimental methods that allow the faster collection of these data sets, often exploiting the improved sensitivity afforded by modern instrument developments, including cryogenic probes. These approaches can be broadly classified as utilizing enhanced instrument hardware capabilities, novel pulse sequence design, modified data sampling schemes, or combinations of these. Improvements in instrument capabilities have provided for "ultra-fast" spectroscopy[2] which exploits the spatial encoding of NMR responses to provide a complete correlation data set in a single-scan. These have also led to the introduction of parallel acquisition NMR (PANSY)[3] using multiple receivers for the simultaneous detection of multiple nuclei and hence multiple correlation spectra. It has been demonstrated that molecular structure of small organic molecules can be established from just a single sensitivity demanding experiment (PANACEA)[4] using multiple receivers. The use of interleaved acquisition allows recording spectra from several uncoupled nuclear species in a similar fashion.[5] However, due in part to the specific hardware requirements and low sensitivity, the ultrafast and multi-receiver approaches so far remain uncommon.

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Supporting information for this article is given via a link at the end of the document.

Here we show that as many as five conventional NMR pulse sequences based on ^{1}H direct detection can be combined into a single supersequence that requires only a single receiver. This approach offers significant time savings and increases the efficiency of NMR experiments as compared to conventional data recording since only a single recovery (relaxation) delay (d_1) is employed in the combined pulse sequences.

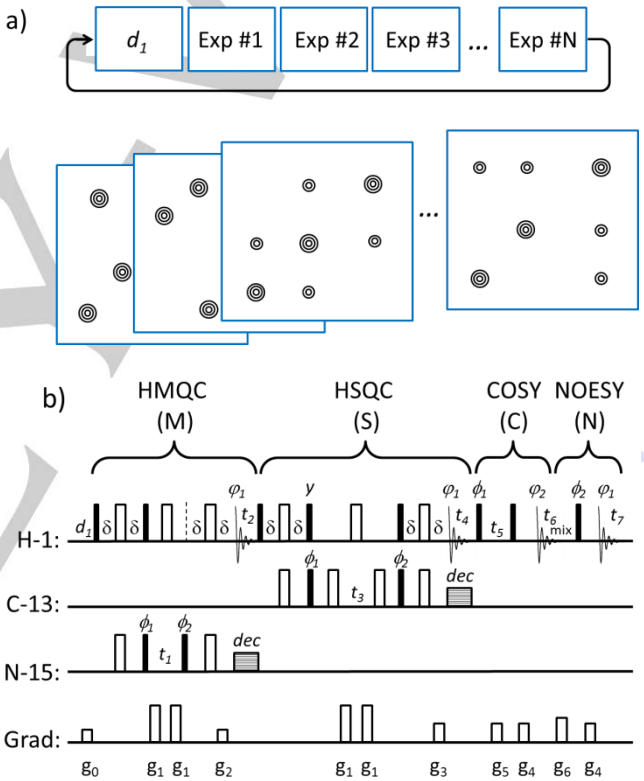

Figure 1. a) A schematic representation of the nested (NOAH) supersequences. Only a single recovery delay, d_1, is employed for up to N=5 nested sequences leading to significant time savings; b) The NOAH-4 (MSCN) pulse sequence combining 2D ^{15}N HMQC, 2D ^{13}C HSQC (multiplicity editing optional; see Fig S1a), 2D ^{1}H-^{1}H COSY and 2D ^{1}H-^{1}H NOESY experiments. All pulses are applied with phase *x* unless indicated otherwise (filled= 90° and open= 180° pulses). Phase cycles: ϕ_1=x,-x, ϕ_2=x,x,-x,-x, receiver phases: φ_1=x,-x,-x,x, φ_2=x,-x; The δ delays are the *J*-evolution delays set to $1/4J_{NH}$ and $1/4J_{CH}$ in the HMQC and HSQC modules respectively, *mix* is the NOESY mixing delay and d_1 is the common recovery delay; gradients (ms, G/cm): g_0=(1,7), g_1=(1,40), g_2=(1,8.1), g_3=(1,20.1), g_4=g_5 (1,20), g_6=(1,17). The polarity of gradient pulses, g_1, g_4 and all receiver phases are inverted for all even increments. The 180 degree ^{13}C pulses are constant adiabaticity WURST pulses.

The proposed technique is outlined schematically in Figure 1a and combines several previously introduced approaches, as

discussed in detail below. Specifically tailored pulse sequences have been developed previously to reduce the duration of data acquisition by minimizing the recovery delay between repeated transients, as exemplified in the *acceleration by sharing adjacent polarization* (ASAP) scheme.[6] This exploits the concept of polarization sharing in which a stored reservoir of magnetization of ^{12}C-bound passive protons is used to replenish the magnetization of the ^{13}C-bound active protons that are used to generate a ^{1}H-^{13}C heteronuclear correlation spectrum. This may be achieved either through the use of NOE transfer[7] or a short (< 50 ms) homonuclear isotropic mixing scheme[6] to pass magnetization from the passive reservoir to the active protons. This short mixing period is used in place of the conventional recovery delay (d_1, typically at least 1 s), so reducing significantly the time required to record a heteronuclear correlation spectrum.

In this work we further exploit the concept of tailored polarisation storage by recording multiple two-dimensional data sets nested within a single experiment for greatly accelerated data collection. This utilises multiple-FID acquisitions per measurement (see Figure 1), with each designed to optimally utilise coherences from the various reservoirs of proton magnetization within a molecule. The sequences presented incorporate multiple heteronuclear and homonuclear correlations with each acquisition based on ^{1}H detection to provide optimum sensitivity. We term this concept NOAH (***N**MR by **O**rdered **A**cquisition using ^{1}**H**-detection*).

The notion of nested sequences was previously introduced long ago with the COCONOESY experiment[8] which yielded separate 2D COSY and NOESY spectra from the same experiment. In this, both 2D experiments share the same recovery delay, d_1 and evolution period, t_1 with the COSY acquisition occurring within the NOESY mixing time, thus providing the two experiments in the time required for NOESY alone. Similar experiments for recording two spectra simultaneously have been proposed in labeled protein samples both in liquids[9] and solids.[10] These should be distinguished from time-shared experiments[11] that collect the same type of spectra from different nuclear species, usually ^{15}N and ^{13}C, and require doubling the number of scans for each additional spectrum to allow for phase encoding.

With the NOAH sequences, we demonstrate the nesting of up to five 2D correlation experiments (NOAH modules), including various combinations of the established methods for small-molecule characterization mentioned earlier. We suggest that hundreds of such combinations are possible (see Table S1 in the Supporting Information). By analogy to the nested phase cycles that are commonly known as *supercycles* we call such nested pulse sequences NMR *supersequences*.

We illustrate the concept with one of the several possible implementations of the NOAH-4 supersequences (see Figure 1b). The pulse sequence starts with the recovery delay, d_1 that typically is by far the longest pulse sequence event. Having a single recovery delay for all four sequences in this NOAH-4 supersequence greatly reduces the experiment duration and improves the efficiency of precious NMR system usage. This is achieved by preserving the coherences of interest largely undisturbed for the consecutive modules throughout the supersequence. Following the recovery delay (d_1) the experiment begins with the least sensitive module, ^{1}H-^{15}N HMQC. Just like in the ASAP HMQC experiment[6] this module ensures that the bulk magnetization of protons that are not directly bound to ^{15}N is preserved for the subsequent NOAH sequence modules by keeping it along the +z-axis. Only 0.37 % of the total proton magnetization is used for this HMQC module. This is followed by the ^{13}C HSQC pulse sequence where the phase cycle and gradients are arranged in such a way as to keep the magnetization of protons that are not directly coupled to ^{13}C undisturbed along the +z axis. A further 1.1% of the total ^{1}H magetization is used by this module. The remaining ^{1}H magnetization is then split equally for recording the two ^{1}H-^{1}H correlated experiments, COSY and NOESY spectra. These two modules are incorporated into the NOAH-4 supersequence according to the COCONOSY scheme.[8] At the end of the t_5 (COSY t_1) evolution period a 90˚ proton read pulse transfers the frequency encoded magnetization to the coupled proton sites. Following this read pulse half of the magnetization is stored along the z axis while the other half is refocused by the decoding gradient and observed during the free induction decay, t_6 providing the 2D COSY spectrum. The COSY acquisition period, t_6 is incorporated into the NOESY mixing period. Both the COSY and NOESY experiments in this NOAH-4 pulse sequence share the same t_1 evolution period (t_5). The COSY acquisition period has appended a delay (*mix*) to meet the requirements of the total duration of the NOESY mixing period (t_6 + *mix*). The NOESY module ends with a read pulse and a decoding gradient before the 2D NOESY spectrum is acquired (t_7). Thus all four 2D spectra in this version of the NOAH-4 experiment are recorded starting from a single d_1 recovery delay.

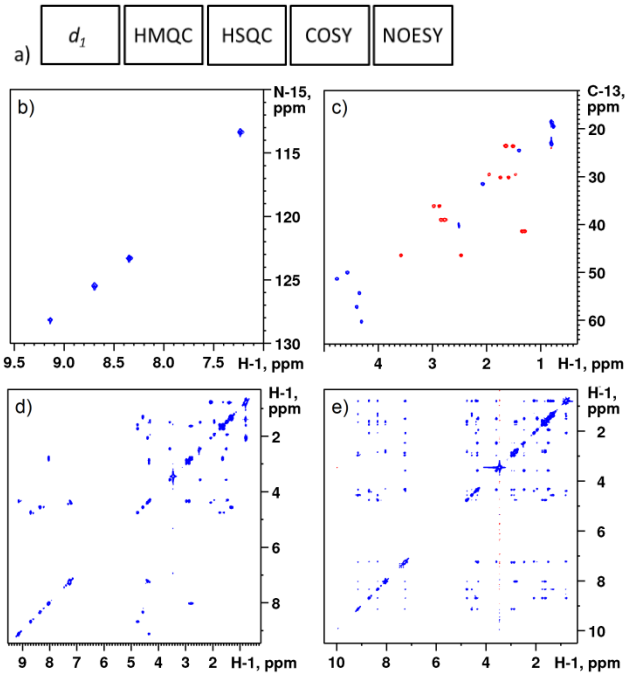

Figure 2. A schematic representation of the NOAH-4 supersequence, MSCN (a) and the 2D spectra recorded in a single experiment, (b) ^{1}H-^{15}N HMQC, (c) multiplicity edited ^{1}H-^{13}C HSQC, (d) ^{1}H-^{1}H COSY and (e) ^{1}H-^{1}H NOESY. The sample is 50 mM gramicidin S in DMSO-*d6*. The spectra were recorded on an AVANCE III spectrometer equipped with a TCI Cryoprobe in 38' 26'' with 2

scans per increment and 512 t_1 increments per module resulting in 2k x 2k raw data matrix. Further experimental details and a comparison between NOAH and conventional data can be found in the Supporting Information.

The NOAH-4 supersequence, MSCN, produces four 2D spectra in one measurement - ^{1}H-^{15}N H**M**QC (M), ^{1}H-^{13}C H**S**QC (S) with optional multiplicity editing, **C**OSY (C) and **N**OESY (N) with the highlighted single letter abbreviations used to identify each module indicated in the parenthesis (Figure 2). Considering that in most conventional experiments and in small molecule experiments in particular the recovery delay, d_1 is typically by far the most time consuming part of the experiment, the time savings provided by the NOAH supersequences are substantial and increase as more modules are combined into a single supersequence (see Figures S2 and S3 for a comparison with conventionally recorded data).

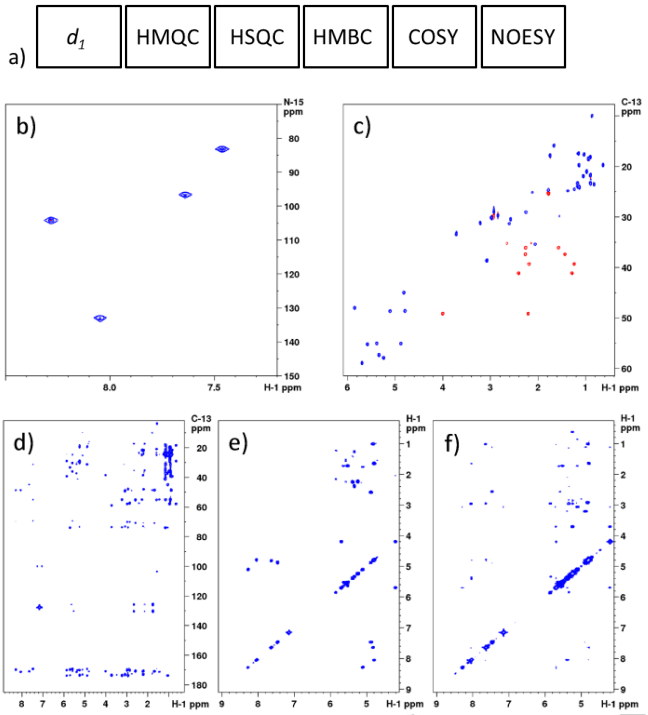

Figure 3. A schematic representation of the MSBCN NOAH-5 supersequence (a) and the 2D spectra recorded in a single experiment, (b) ^{1}H-^{15}N HMQC, (c) multiplicity edited ^{1}H-^{13}C HSQC, (d) ^{1}H-^{13}C HMBC, (e) ^{1}H-^{1}H COSY and (f) ^{1}H-^{1}H NOESY. The sample is 50 mM cyclosporine in benzene-*d6*. The spectra were recorded on an AVANCE III spectrometer equipped with a TCI CryoProbe in 44 minutes with 2 scans per increment and 512 t_1 increments per module resulting in 2k x 2560 raw data matrix.

The second example demonstrates one possibility of the NOAH-5 supersequence, MSBCN that combines ^{1}H-^{15}N H**M**QC (M), multiplicity edited ^{1}H-^{13}C H**S**QC (S), ^{1}H-^{13}C HM**B**C (B), **C**OSY (C) and **N**OESY (N) pulse sequences. The experiment is similar to the NOAH-4 supersequence, MSCN except the HMBC module is incorporated between the HSQC and COSY modules. This NOAH-5 supersequence (Figure S1b), produces five 2D spectra in one experiment (Figure 3) delivering all the information that is required for small molecule structure elucidation in a single measurement. The technical aspects of this pulse sequence are described in more detail in the Supporting Information.

Further examples of NOAH style supersequences for applications to samples at natural isotopic abundance are listed in Tables 1 and S1 (see the Supporting Information) and illustrative spectra are provided in Figures S2-S11. These include (but not in any way limited to) seven of the most frequently used 2D NMR experiments – H**S**QC (S), H**M**QC (M), HM**B**C (B), **C**OSY (C), **D**QF-COSY (D), **T**OCSY (T) and **N**OESY (N). The efficiency of the NOAH type supersequences can be further improved by combining these experiments with other fast techniques, such as non-uniform sampling,[12] Hadamard spectroscopy,[13] projection spectroscopy,[14] use of multiple receivers[3] and similar. The utility of the NOAH supersequences has been successfully tested on multiple NMR systems in our laboratory. While not all of the 285 NOAH supersequences listed herein and in the Supporting Information are equally efficient and/or practical, the existence of the large number of possible combinations highlights the need for a systematic classification of this technique, and a more complete description of their design and operation will be provided in future work. While here we only have considered two-dimensional experiments, preliminary work suggests that similar extensions to three and higher dimensional experiments are possible.[9a,10]

Table 1. Selected examples of useful combinations of NOAH 2D supersequences. Shaded rows indicate those utilising three rf channels (^{1}H/^{13}C/^{15}N); all others require only two (^{1}H/X). Over 250 further supersequence combinations are listed in the Supporting Information.

No	Exp 1	Exp 2	Exp 3	Exp 4	NOAH code
1	^{13}C HSQC	COSY	-	-	SC
2	^{13}C HSQC	NOESY	-	-	SN
3	^{13}C HSQC	TOCSY	-	-	ST
4	^{13}C HSQC	DQFCOSY	-	-	SD
5	^{13}C HSQC	^{13}C HMBC	-	-	SB
6 (Fig.S9)	^{13}C HMQC	COSY	-	-	MC
7	^{13}C HMQC	NOESY	-	-	MN
8	^{13}C HMQC	TOCSY	-	-	MT
9 (Fig.S10)	^{13}C HMQC	^{13}C HMBC	-	-	MB
10	^{13}C HMQC	NOESY	-	-	MN
11	COSY	NOESY	-	-	CN
12	^{15}N HMQC	^{13}C HSQC	COSY	-	MSC
13	^{15}N HMQC	^{13}C HSQC	NOESY	-	MSN
14	^{15}N HMQC	^{13}C HSQC	TOCSY	-	MST
15	^{15}N HMQC	^{13}C HSQC	DQFCOSY	-	MSD
16	^{15}N HMQC	^{13}C HSQC	^{13}C HMBC	-	MSB
17	^{15}N HMQC	COSY	NOESY	-	MCN
18	^{13}C HMQC	^{13}C HMBC	COSY	-	MBC
19	^{13}C HMQC	^{13}C HMBC	DQFCOSY	-	MBD
20	^{13}C HSQC	COSY	NOESY	-	SCN
21	^{13}C HSQC	^{13}C HMBC	COSY	-	SBC
22	^{13}C HSQC	^{13}C HMBC	DQFCOSY	-	SBD
23	^{15}N HMQC	^{13}C HSQC	COSY	NOESY	MSCN
24	^{15}N HMQC	^{13}C HSQC	^{13}C HMBC	COSY	MSBC

Experimental Section

All spectra were recorded on a Bruker AVANCE III NMR spectrometer operating at 700 MHz ^{1}H frequency and equipped with a TCI CryoProbe optimized for ^{1}H detection. Further tests (not shown here) were carried out on a Bruker AVANCE III NMR spectrometer operating at 500 MHz ^{1}H

frequency and equipped with a BBFO SMART probe. Three simple additional processing routines (au-programs) were written for automatic data separation (splitx) and the frequency axis adjustment for homonuclear experiments (fixF1) or ^{15}N heteronuclear experiments (fixF1n) following data separation into individual data subsets. These routines along with the NOAH-4 and -5 pulse programs are provided in the SI. Further sequences are available from the authors upon request and will also be available from the Bruker online User Library.

Keywords: NMR Spectroscopy • Structure Elucidation • NOAH • HSQC • COSY

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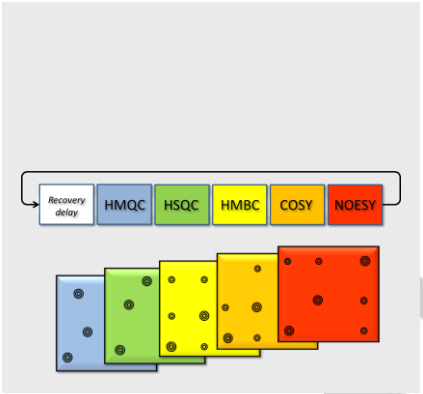
COMMUNICATION

NOAH's Ark:

The nesting of up to five conventional NMR pulse sequences into one "NOAH supersequence" employing only a single recovery delay leads to significant time savings in data collection for the structure elucidation of small molecules. The experiments operate without the need for specialist spectrometer hardware.



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