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New NOAH modules for structure elucidation at natural isotopic abundance

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ABSTRACT

We introduce several new NOAH modules designed for NMR supersequences that allow structure elucidation of small organic molecules from a single measurement. We show that double isotope filters (*ZZ*-filters) increase the flexibility of module permutation within the NMR supersequences, optimising combinations exploiting ^{15}N and ^{13}C nuclides. The time-shared 2BOB module combined with the ZZ-HMBC module (yielding NOAH-2 BO) provides an example of extending the NMR supersequences with parallel experiments (here 2BOB) that are incompatible with sequential implementation. Finally, the PANSY-COSY module combined with the HSQC sequence (yielding NOAH-2 SC^2) provides an example of incorporating multiple receiver experiments into NMR supersequences opening new avenues for designing information rich NMR experiments. The new NOAH supersequences were utilized in computer assisted structure elucidation (CASE) study accomplished using the CMCse software.

1. Introduction

Structure elucidation by NMR [1–3] is heavily based on the (scalar) *J*-coupling networks between spin-1/2 nuclei exploited in homonuclear and heteronuclear correlation methods [4]. Typically, one-bond correlation techniques provide direct connectivity information, long-range correlations connect molecular fragments and through-space dipolar couplings provide supplementary stereochemical (3D) information [5–7]. Three basic NMR experiments – HSQC, HMBC and COSY are usually employed to obtain information about one-bond X-H (HSQC), long range X-H (HMBC) and three-bond H-H (COSY) connectivities (X = ^{13}C and ^{15}N). Additional experiments, such as NOESY, ROESY, TOCSY, DQF-COSY, HMQC, H2BC, HSQC-TOCSY and occasionally 1,1-ADEQUATE (or its variants) are also used to obtain complementary information when the basic experiments prove to be insufficient for structure elucidation purposes.

Many of these experiments can be combined into NMR supersequences that provide the required structural information in a single measurement, saving considerable time. For instance, the PANACEA (**P**arallel **A**cquisition **N**MR, an **A**ll-in-one **C**ombination of **E**xperimental **A**pplications) experiment [8–10] combines the INADEQUATE, HSQC and HMBC pulse sequences into a single entity (a supersequence) that allows structure elucidation from a single measurement. While the INADEQUATE experiment in the PANACEA pulse scheme is one of the most powerful techniques for structure elucidation of small molecules, at natural isotopic abundance it is also one of the least sensitive small molecule NMR experiments. Alternatively, the NOAH (NMR by Ordered Acquisition using ^{1}H detection) supersequences [11–13] are constructed from the basic and more sensitive ^{1}H-detected experiments (modules) that are typically used for small molecule analysis and structure elucidation. Such supersequences not only provide significant time savings and often a superior sensitivity per unit time, but they also ensure that all spectra are recorded under identical experimental conditions thus eliminating inter-experiment variability and associated signal assignment ambiguities.

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The considerable time savings provided by the NOAH type supersequences are achieved because these experiments require only a single recovery period, d_1 for recording several NMR spectra. For brevity the NOAH modules are assigned a single letter, *e.g.* S (for HSQC), B (HMBC), Q (HMQC), C (COSY), N (NOESY), R (ROESY) and similar [11]. While hundreds of NOAH-type combinations are possible [11], rather fewer NOAH supersequences, including BSC, BSCN, SBC, and SBCN, provide sufficient information in a single measurement to allow computer assisted structure elucidation (CASE) [12]. Here we introduce new NOAH modules based on the principles of isotope filtering [14], preservation of equivalent coherence pathways [15,16], time-sharing [17–19] and multiple receiver techniques [20,21]. These enable the design of new and efficient supersequences for fast structure elucidation of small organic molecules from a single measurement in conjunction with the CASE techniques. The proposed pulse schemes achieve significant time savings in situations where the sensitivity is sufficient to record the least sensitive conventional experiments with just a few scans per increment. Furthermore, we show that this translates into improvements in sensitivity per unit time.

2. Results and discussion

The basic set of experiments that are generally employed for structure elucidation of small organic molecules include ^{13}C-^{1}H HSQC, ^{13}C-^{1}H HMBC and ^{1}H-^{1}H COSY. Therefore, the NOAH-3 SBC and the favoured BSC supersequences can be used for structure elucidation in a single measurement. The BSC configuration has the advantage of maximizing the sensitivity of the HMBC module (B) and reducing the T_2 relaxation losses in the COSY module (C) [12]. Further information about the spatial structure of molecules can be obtained by incorporating the NOESY and ROESY modules that result in BSCN and BSCR type supersequences. Nitrogen containing molecules may also require ^{1}H-^{15}N correlation experiments to provide important information about the position of the nitrogen atoms in the molecule. For this, the ^{15}N HMQC module is preferred to ^{15}N HSQC because the former handles the bulk magnetization more efficiently and in N-H correlated spectra the multiplicity information is typically not needed. While prepending the ^{15}N HMQC module (M) to the NOAH-3 SBC supersequence (yielding NOAH-4 MSBC) is trivial, [11] it is not quite as straightforward in the case of BSX (X = C, N, R and similar) type of NOAH-3 supersequences (*vide infra*). Here we introduce a dual H-C/H-N filter to allow construction of the NOAH BMSC based supersequences.

Whilst the MBSC configuration of the NOAH modules is possible, it is advantageous to start the NOAH supersequences with the least sensitive experiment. Although the ^{15}N HMQC is by a factor of ~3 less sensitive as compared to an equivalent ^{13}C experiment, the ^{13}C HMBC often contains weak signals of interest due to the wide range of $^{n}J(C\text{-}H)$ ($n > 1$) coupling constants and therefore we prefer to start our supersequence with the ^{13}C HMBC module. This requires a dual *J*-filter that preserves the one-bond ^{1}H-^{13}C and ^{1}H-^{15}N magnetization prior to the HMBC module. The NOAH-4 BMSC pulse sequence and the dual ZZ filter are shown in Fig. 1. Note that the ^{15}N HMQC module (Fig. S1) is designed to preserve the one-bond ^{1}H-^{13}C magnetization that is utilized in the subsequent ^{13}C HSQC module [11].

The semi-adiabatic double ^{1}J-filter features a *J*-compensated adiabatic WURST pulse on ^{13}C channel [22,23] while the less demanding bandwidth requirements of ^{15}N nuclei can be adequately addressed by the conventional hard π pulse. Since the $^{1}J(CH)$ and $^{1}J(NH)$ couplings are quite different (typically ca 140 and 90 Hz) an appropriate displacement of the ^{13}C and ^{15}N π pulses is required for optimal performance. However, the duration of the

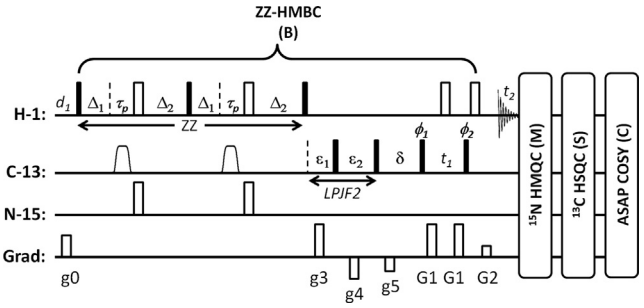


Fig. 1. The NOAH-4 BMSC supersequence showing explicitly the ZZ-HMBC module and schematic representation of the HMQC (M), HSQC (S) and ASAP COSY (C) modules described in [12] and in the Supporting Information; the ZZ-HMBC module includes semi-adiabatic double ^{13}C/^{15}N ^{1}J-filter and the second-order low-pass ^{13}C ^{1}J-filter, *LPJF2* [24] for better suppression of the one-bond C-H correlations in the ^{13}C HMBC spectra; filled rectangles denote $\pi/2$ pulses and hollow rectangles denote π pulses; the shaped pulses are 1.58 *ms* long adiabatic WURST pulses covering 340 *ppm* bandwidth with sweep direction from low to high field; all pulses are applied with phase *x* unless indicated otherwise; phases: $\phi_1 = x, -x, \phi_2 = x, x, -x, -x$, $rec = x, -x, -x, x$; delays: $\Delta_1 = 0.5/^{1}J_{av}(CH) + \tau_p/2$, $\Delta_2 = 0.5/^{1}J_{av}(CH) - \tau_p/2$, where τ_p is the duration of the adiabatic WURST pulse, $\delta = 0.5/^{n}J(CH)$, $\varepsilon_1 = 0.5/^{1}J_{min}$, $\varepsilon_2 = 0.5/^{1}J_{max}$; gradients (G/cm, ms): $g0 = (16.5, 1.0)$, $g3 = (7.5, 1.0)$, $g4 = (-5, 1.0)$, $g5 = (-2.5, 1.0)$, the coherence selection gradients $G1 = (40, 1.0)$, $G2 = (20.05, 1.0)$; the polarity of the gradient pulses *G1* and the receiver phase were inverted for all even increments.

^{13}C pulse is such that to a good approximation no further arrangement of the two π pulses is necessary thus simplifying implementation of this double $^{1}J(C\text{-}H/N\text{-}H)$ filter (see Fig. 1). The double ^{1}J -filter is followed by the HMBC module described previously [11], except a further second order low pass ^{1}J-filter [24] is introduced in the HMBC module for better suppression of the one-bond C-H -correlations in the HMBC spectra. The effects of the ZZ-filter on the sensitivity of the HMBC module are minor [13]. The rest of the pulse sequence is a straightforward adaptation of the NOAH MSCX supersequences (X = N or R). The spectra of antihypertensive drug ajmalicine recorded using the NOAH-4 BMSC supersequence are shown in Fig. 2 along with the CASE structure of the molecule solved using the CMCse software [25,26].

It is worth noting that the ^{13}C HMBC module can be replaced with simultaneous ^{15}N and ^{13}C HMBC experiments that can be recorded in a time-shared manner [27]. However, due to small and often unpredictable long-range H-N couplings coupled to the low natural abundance of the ^{15}N isotope, the ^{15}N HMBC experiment is typically of considerably lower sensitivity as compared to the rest of NOAH modules in these series. Furthermore, the unpredictability of the long-range $^{n}J(N\text{-}H)$ couplings often requires the recording of several spectra with different $^{n}J(N\text{-}H)$ settings. Therefore, it was deemed that the time-shared C/N HMBC module would be impractical for our purposes.

While recording the one-bond N-H and C-H correlation spectra in time-shared manner is also possible [17,19], if not somewhat more cumbersome, the sequential arrangement used here has a slight advantage of providing a better resolution, as is shown in the next example (see Scheme 1).

Previously we have demonstrated that the structure of small organic molecules can be derived from the NOAH-3 BSC experiment [12]. However, for crowded ^{1}H spectra the CASE study based on this type of NOAH supersequences may fail. The problem of overlap in proton spectra is typically resolved by recording H-H correlated spectra that are resolved in the F_1 dimension that involves one of the spin-1/2 heteronuclei, typically ^{13}C or ^{15}N. For instance, HSQC-TOCSY, HMQC-TOCSY, HSQC-COSY, HMQC-COSY [28] and their derivatives, such as H2BC [29] and 2BOB [30] can be employed. However, these experiments are not easily incorporated into the NOAH-type supersequences because the magnetization of protons directly bound to ^{13}C is also required by the HSQC (or HMQC) modules.

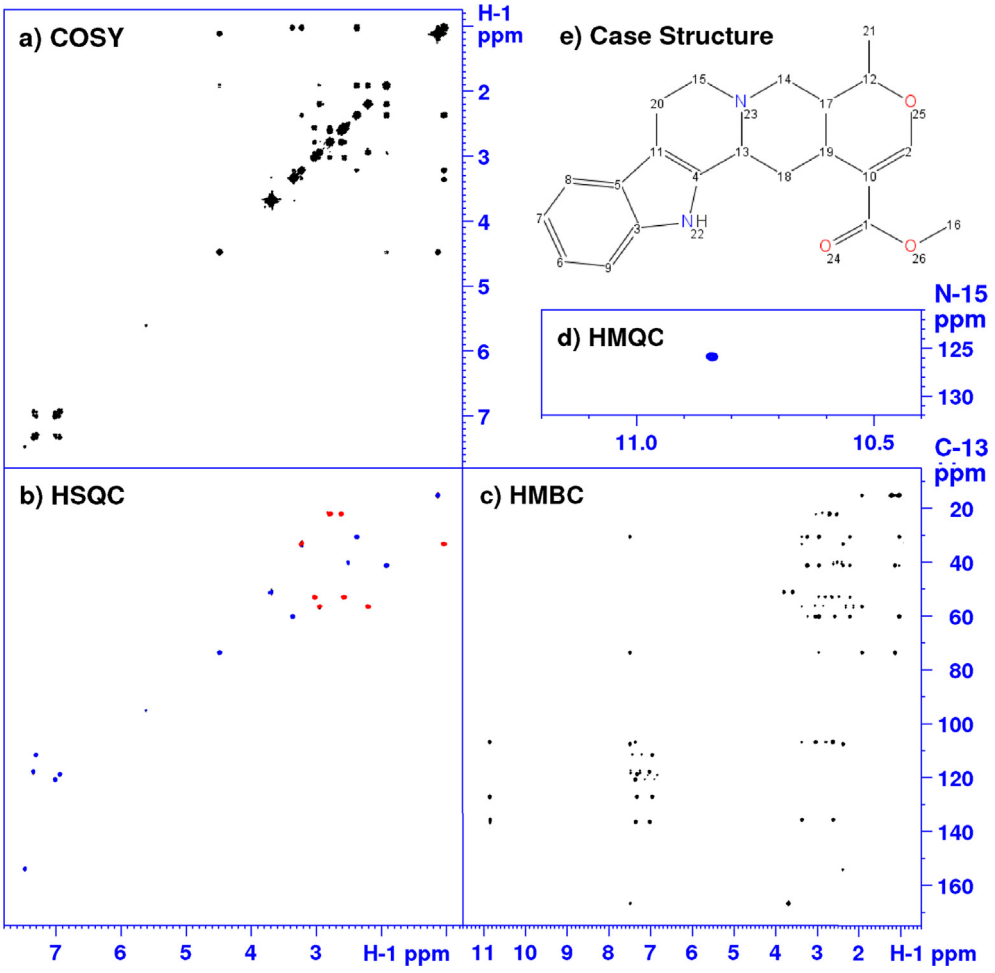
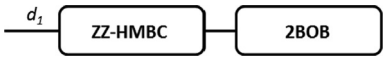


Fig. 2. (a)–(d) spectra of 23 mM ajmalicine in DMSO-d_6 recorded in 19 min. 48 sec. on a 700 MHz Avance III HD spectrometer equipped with a TCI helium cryoprobe using the NOAH-4 BMSC pulse sequence of Fig. 1; 2 scans per increment, 16 dummy scans, recovery delay d_1 = 1.5 *s*, the raw data size was 2 k × 1 k (2 k × 256 per module), spectral width was 8 kHz (^{1}H, ^{15}N) and 32 kHz (^{13}C); the combined duration of equivalent individual experiments was 59 min. 24 sec. (e) the CASE structure obtained with the CMCse software.



Scheme 1. Schematic representation of the NOAH-2 BO pulse sequence consisting of ZZ-HMBC (see Fig. 1) and 2BOB modules (described in [30] and in the Supporting Information).

The 2BOB (**T**wo-**B**ond, **O**ne-**B**ond correlation experiment) pulse scheme [30] that is derived from the H2BC (**H**eteronuclear **2**-**B**ond **C**orrelation) sequence records both, one-bond H-C and two-bond H-H-C correlations in a time-shared manner. The H2BC experiment can be seen as a derivative of the HMQC-COSY pulse scheme providing two-bond H-H-C correlations. The multiplicity edited one-bond C-H correlations that are preserved by the 2BOB pulse scheme in the concurrent constant time (CT) HMQC experiment are recorded in parallel. The time-sharing technique [19] allows the separation of the two sub-spectra (H2BC and CT-HMQC), but requires at least two scans per t_1 increment to allow for phase encoding of the two sub-spectra. Combined with the HMBC module, such a NOAH-2 BO supersequence (O denotes the 2BOB module) provides the required information for structure elucidation of small molecules (see scheme 1). The spectral resolution in the HMBC spectra can be doubled by recording twice as many time increments as in the time-shared 2BOB module. This indicates that sequential implementation is preferable when possible.

The separation of the H2BC and multiplicity edited CT-HMQC spectra provides additional simplification in spin systems with severe ^{1}H signal overlap. The multiplicity editing step can be used to separate the CH_2 peaks from the CH and CH_3 peaks to provide an option for a further spectrum simplification [30]. This, however, increases the minimum number of steps per t_1 increment to four. Alternatively, in less severe peak overlap situations, both the multiplicity editing and the two-bond and one-bond correlation information can be obtained from the peak line-shape and phase information in a single step, thus avoiding the need for the time sharing step [30].

The spectra of gibberellic acid (GA3, hormone found in plants and fungi) dissolved in DMSO-d_6 are shown in Fig. 3 along with the structure obtained in CASE study using the CMCse software. Previously our efforts to obtain the structure of GA3 in DMSO-d_6 from the NOAH-3 BSC spectra recorded on a 700 MHz instrument failed because of the severe overlap in the COSY spectra and the structure was instead obtained from the sample dissolved in acetone-d_6 [12]. The 2BOB module provided the required spectral resolution for structure elucidation from the sample dissolved in more commonly used DMSO-d_6. Therefore the NOAH-2 (BO) experiment achieves a significant time saving (78%) which translates into improvements in sensitivity per unit time in comparison to the corresponding standalone experiments (see the Supporting Information).

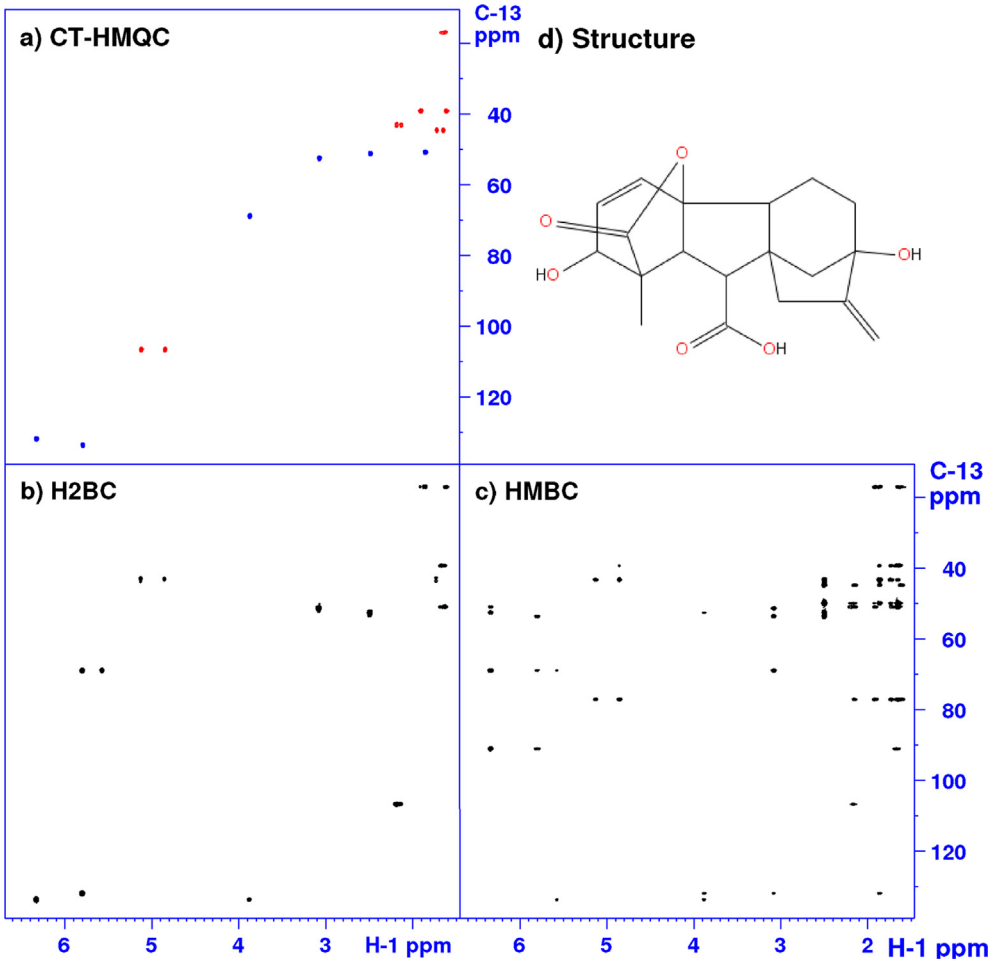


Fig. 3. (a)–(c) The NOAH-2 BO spectra of gibberellic acid 20 mM solution in DMSO-d_6 recorded on a 700 MHz Avance III HD spectrometer in 17 min. 34 sec. using the NOAH-2 BO supersequence shown in Scheme 1; raw data size was 2 k × 512 (2 k × 256 per module), 2 scans per increment, 4 dummy scans, spectral width was 6250 Hz (^{1}H) and 32 kHz (^{13}C) (d) the CASE structure obtained with the CMCse software.

Typically the multiplicity edited ^{13}C-^{1}H HSQC spectra provide one-bond C-H connectivities and the multiplicity information, the ^{1}H-^{1}H COSY spectra provide three-bond H-C-C-H connectivities and the ^{13}C-^{1}H HMBC spectra allow linking molecular fragments that are not connected via the H-H coupling networks. In principle, all this information can be obtained in a single 2D experiment – the dual receiver HH/HC PANSY-COSY [20]. Due to substantially better signal dispersion over the wide spectral bandwidth in ^{13}C spectra as compared to ^{1}H spectra the experiments with direct ^{13}C detection benefit from significantly better ^{13}C resolution and at the same time require considerably smaller number of time increments in the indirect (^{1}H) dimension, but suffer from low sensitivity. Furthermore, the PANSY-COSY experiment is not very practical because the ^{1}H-^{13}C COSY spectra contain complex peak patterns due to one-bond ^{13}C-^{1}H couplings that are present in both, F_1 and F_2 dimensions thus complicating the peak assignment and further reducing the signal-to-noise ratio (S/N). However, the long range correlations to non-protonated carbon atoms in the ^{1}H-^{13}C COSY spectra do not suffer from this deficiency and the PANSY-COSY module can be useful as a replacement for the HMBC module, as shown in the next example.

A simple substitution of the COSY module in the NOAH-2 SC supersequence with the PANSY-COSY pulse scheme leads to the NOAH-2 SC^2 supersequence where C^2 denotes the dual-receiver PANSY (**P**arallel **A**cquisition **N**MR **S**pectroscop**Y**) COSY module (see Fig. 4a). Thus, the C^2 module provides both the ^{1}H-^{1}H COSY and the long-range ^{1}H-^{13}C correlation spectra while the one-bond H-C correlations are more efficiently obtained from the preceding HSQC module. The cross-peak patterns in the F_1 domain of the ^{1}H-^{13}C COSY spectra are simplified by suppressing the one-bond C-H correlations in the C^2 module by the ^1J-filter (see Fig. 4a). While the $^1J(CH)$ splittings are still present in the direct dimension of the ^{1}H-^{13}C COSY spectra the coupling patterns are greatly simplified and their assignment is facilitated by the simultaneously acquired HSQC spectra. The main objective of the ^{1}H-^{13}C COSY spectra is to provide long-range H-C connectivities to the non-protonated carbons that do not suffer from the one-bond C-H splittings. The spectra of pamoic acid recorded with the NOAH-2 SC^2 supersequence are shown in Fig. 5. The NOAH-2 SC^2 supersequence serves as a simple example of augmenting the NOAH supersequences with multi-receiver modules and the possibility of branching the coherence transfer pathways with simultaneous detection of nuclei other than protons, such as ^{13}C, ^{19}F, ^{31}P and similar.

Although the NOAH-2 SC^2 is the shortest NOAH-type supersequence that allows structure elucidation from a single experiment it does require relatively high sample concentrations and/or high sensitivity ^{13}C-observe CryoProbes. High gamma and high natural abundance hetero-nuclei, such as ^{19}F and ^{31}P provide further opportunities for augmenting the NOAH supersequences with multi-receiver experiments of comparable sensitivity [31]. One such example is shown in Fig. 4b. This pulse scheme combines

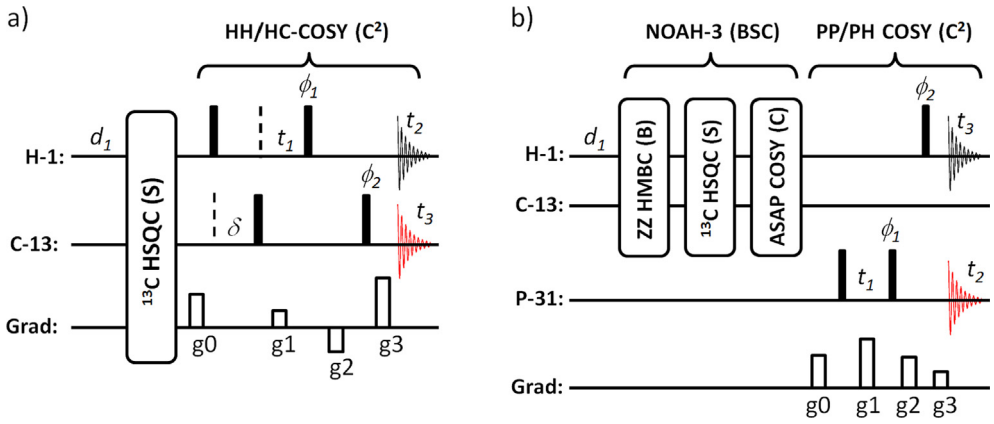


Fig. 4. The NOAH supersequences augmented with multiple receiver modules; (a) NOAH-2 SC^2 supersequence consisting of the previously published ^{13}C HSQC module [11] that is shown schematically and the parallel HH/HC COSY module (PANSY-COSY [20]) that requires two receivers; all pulses are applied with phase *x* unless specified otherwise; phases: ϕ_1 = *rec1* = *rec2* = *x*, −*x*; delays: $\delta = 0.5/{}^1J_{av}(CH)$; gradient ratios for coherence selection: g3/g1 = γ_H/γ_C, g1 = g2 + g3 gradients (G/cm, ms): *g0* = (17.1, 1.0), *g1* = (7.5, 1.0), *g2* = (−22.5, 1.0), *g3* = (30, 1.0); the polarity of the gradient pulse *g1* and the receiver phase were inverted for all even increments; (b) NOAH-4 BSCC^2 supersequence where the NOAH3-BSC supersequence [12] is augmented with the parallel PP/PH COSY module (PANSY-COSY) that requires two receivers; all pulses are applied with phase *x* unless specified otherwise; phases: ϕ_1 = *rec1* = *rec2* = *x*, −*x*; gradient ratios for coherence selection: g3/g1 = γ_P/γ_H, g1 = g2 + g3; gradients (G/cm, ms): *g0* = (17.1, 1.0), *g1* = (27.5, 1.0), *g2* = (16.4, 1.0), *g3* = (11.1, 1.0); the polarity of the gradient pulse *g1* and the receiver phase were inverted for all even increments.

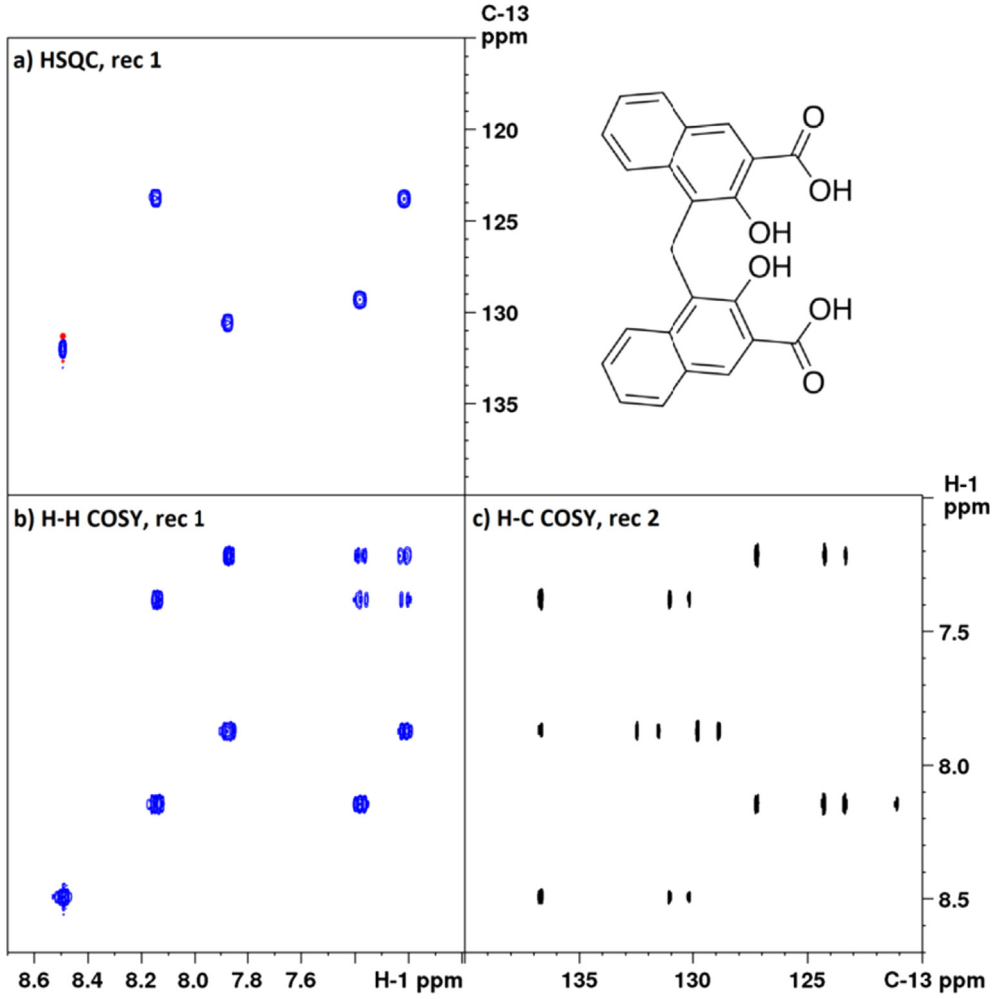


Fig. 5. Spectra (a–c) of pamoic acid, 50 mM in DMSO-d_6 recorded in 7 min. 56 sec. using the NOAH-2 SC^2 supersequence (see Fig. 4a) on a two channel 400 MHz NMR system (Bruker NEO nanobay) equipped with two receivers and a nitrogen cooled BBO Prodigy cryoprobe; the raw data size of the 2 × 16 kHz (^{1}H, ^{13}C) spectral window was 512 × 512 data points (512 × 256 per module), 1 scan per increment, 4 dummy scans, 1.5 *s* recovery delay.

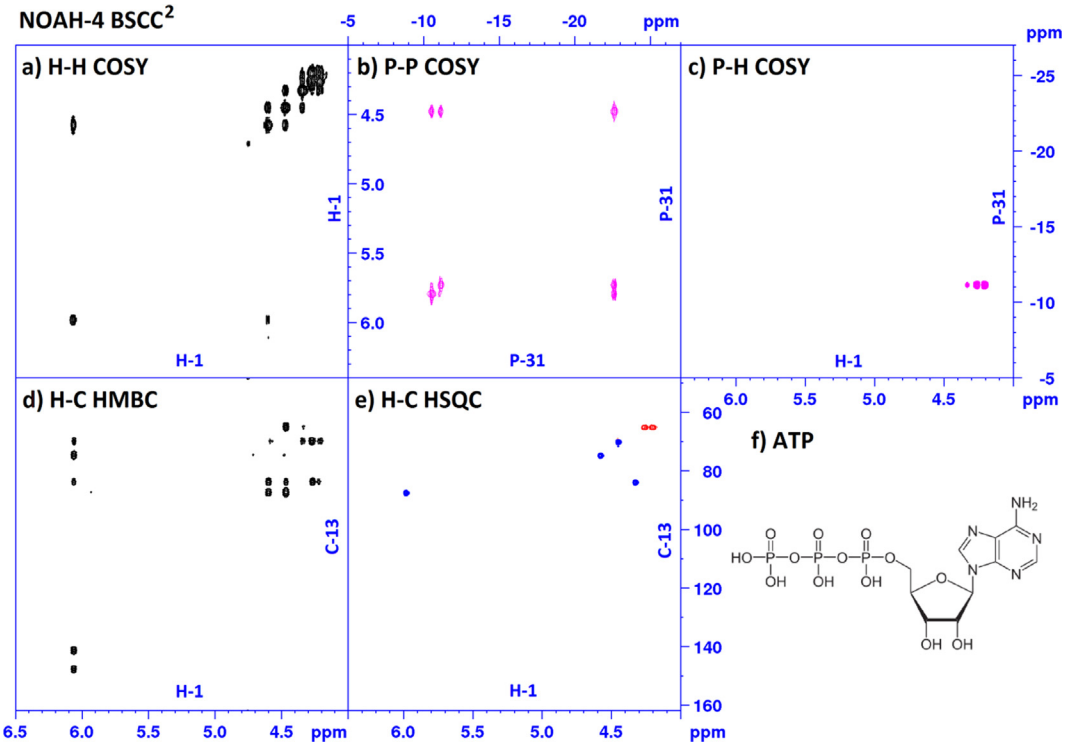


Fig. 6. Spectra (a–e) of the ribose part of ATP, adenosine triphosphate (f), 50 mM in D_2O recorded in 22 min. 51 sec. using the NOAH-4 BSCC^2 supersequence of Fig. 4b on a four channel 700 MHz NMR system (Bruker NEO) equipped with four receivers and a helium cooled QCIP cryoprobe; the raw data size of the 2.5 (^{1}H), 20 kHz (^{13}C) and 6.25 (^{31}P) kHz, spectral windows was 1 k × 1 k data points, 2 scans per increment, 4 dummy scans, 1.5 *s* recovery delay. In the multiplicity edited HSQC spectra negative peaks are shown in red and positive peaks are shown in blue; the correlation spectra involving ^{31}P are shown in pink. The magnitude COSY and HMBC spectra are shown in black.

the NOAH-3 BSC supersequence with the PP/PH PANSY-COSY module leading to the NOAH-4 BSCC^2 supersequence. The PANSY-COSY module records ^{31}P-^{31}P and ^{31}P-^{1}H correlation spectra that reveal additional and important connectivities in phosphorus containing molecules, such as ATP (adenosine triphosphate) as shown in Fig. 6. Although the P-H polarization transfer used here is less efficient when compared to the more commonly exploited H-P transfer, the former uses unperturbed ^{31}P magnetization and the sensitivity of the resulting P-H COSY is still considerably higher as compared to that of the ^{13}C HMBC module.

3. Conclusions

We have introduced two new NOAH modules for structure elucidation of small molecules – the 2BOB (O) and PANSY-COSY (C^2) along with the double semi-adiabatic HC/HN ^1J-filter that extends the functionality of the existing NOAH supersequences. The semi-adiabatic ^1J-filter allows sequential arrangement of the HMBC (B), ^{15}N HMQC (M) and ^{13}C HSQC (S) modules in BMSX (X = other modules) type NOAH supersequences that can be used for structure elucidation. As an example the BMSC supersequence is used in combination with the CMCse software to correctly determine the structure of antihypertensive drug ajmalicine. The NOAH-2 BO supersequence allows structure elucidation in molecules with over-crowded COSY spectra due to the increased resolution in the F_1 (^{13}C) domain. The 2BOB module includes the HMQC-COSY experiment that is recorded simultaneously with the multiplicity edited (me) HMQC spectra in a time-shared mode. The time-shared mode of the 2BOB module allows recording the preceding HMBC module, and more generally any other module(s), with double F_1 resolution. The spectra from the HMBC, me-HMQC and me-HMQC-COSY experiments of the NOAH-2 BO supersequence provided sufficient information for the CMCse software to produce the correct structure of the gibberellic acid in DMSO-d_6.

Finally, we show that the COSY module can be replaced or complemented with the PANSY-COSY module (C^2). This offers ample opportunities for branching the coherence transfer pathways and simultaneous detection of hetero-nuclei such as ^{13}C, ^{19}F and ^{31}P. For instance, where ^{13}C sensitivity permits the two module NOAH-2 SC^2 supersequence can be used for structure elucidation of small molecules in a way that is comparable to NOAH-3 BSC data. Alternatively, the polarization from high gamma nuclei can provide an additional source of magnetization that is typically neglected in the conventional experiments. For example, the NOAH-4 BSCC^2 supersequence produces five spectra in a single measurement – ^{13}C HMBC, ^{13}C HSQC, H-H COSY, X-X COSY and X-H COSY (X = ^{31}P or ^{19}F) revealing, in addition to the H-C connectivities, both the X-X and H-H *J*-coupling networks and connecting them in the same experiment.

In summary, we have demonstrated two new approaches for extending the versatility of the NOAH-type supersequences - (a) the 2BOB experiment represents modules based on time-sharing techniques and (b) the PANSY-COSY experiment represents modules involving multiple receivers. The two major advantages of the NOAH experiments are: (a) the spectra are recorded faster and (b) under identical conditions. The latter is of particular importance for analysis of unstable molecules and environments. Furthermore, in many cases the NOAH technique offers also better sensitivity per unit time as compared to the corresponding standalone experiments (see the Supporting Information). While sequential acquisition of ^{1}H detected experiments is not new [16,27,33–35], it is the modularity aspect of the NOAH technique that allows design of new modules based on a variety of techniques and approaches [32] that can be combined into many different supersequences, which in turn provide more efficient means to

record multiple sets of NMR spectra required for small molecule analysis and structure elucidation.

4. Experimental section

All spectra were recorded on Bruker NEO 400 and 700 MHz systems equipped with the room temperature TCI probe, QCIP (700 MHz) helium and BBO Prodigy (400 MHz) nitrogen CryoProbes at 298 K. The dual receiver $^{13}C/^{1}H$ detected experiments were recorded on the 400 MHz instrument equipped with the BBO Prodigy probe while the $^{31}P/^{1}H$ detected experiments were recorded on the 700 MHz system equipped with the QCIP helium cryoprobe. All other experiments were recorded on the 700 MHz Avance III system equipped with the room temperature TXI probe. The shaped (adiabatic) pulses were generated using the Bruker *WaveMaker* software for optimum performance. All NOAH data sets were processed within *TopSpin* with the splitx_au program (defined by parameter AUNMP) to yield individual processed data sets for each NOAH module. The processing routines are available in the latest releases of the *TopSpin* software or from the Bruker online User Library. The samples of compounds studied here were obtained from Sigma-Aldrich and for the purpose of pulse sequence development were prepared with relatively high concentration of ca 50 mM as 600 μL solutions in DMSO-d_6 in 5 mm sample tubes. Other experimental details were sample dependent and are given in the captions to Figs. 2–6.

Appendix A. Supplementary material

Detailed pulse schemes for the NOAH modules used in this work, sensitivity considerations, complementary spectra, the Bruker pulse programs of the NOAH experiments described in this work and the CMCse structure elucidation reports. Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmr.2019.106568.

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