ChemComm

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *Chem. Commun.*, 2018, **54**, 7139

Received 23rd April 2018,
Accepted 25th May 2018

DOI: 10.1039/c8cc03296c

rsc.li/chemcomm

Molecular structure from a single NMR supersequence†

Ēriks Kupče *[a] and Tim D. W. Claridge *[b]

New NOAH supersequences (NMR by Ordered Acquisition using ^{1}H-detection) are introduced that allow fast structure elucidation of organic molecules from a single measurement. The application of the proposed NOAH-3 BSC and NOAH-4 BSCN experiments, combining two new modules (ZZ-HMBC (B) and ASAP-COSY (C)) with multiplicity-edited HSQC (S) and NOESY (N), is exemplified here through their use in computer-assisted structure elucidation.

The use of optimized approaches for the time-efficient collection of NMR data plays an increasingly important role in the routine characterization of molecular structures. These may include the use of higher sensitivity cryogenic probes,[1] or modified approaches to data collection,[2] and may come to exploit more recent developments in instrument design, such as parallel acquisition using multiple receivers.[3,4] It has been demonstrated that the structure of small organic molecules can be established from a single NMR measurement called PANACEA,[5,6] (akin to single measurement procedures such as X-ray crystallography). However, the PANACEA experiment is based on the ^{13}C–^{13}C INADEQUATE pulse sequence that requires high sample concentration and/or long acquisition times.[7–9] Recently, we described a new class of NMR experiments, the NOAH (NMR by Ordered Acquisition using ^{1}H-detection) supersequences, that are based on ^{1}H detection and allow recording of several two-dimensional (2D) spectra with high sensitivity in a single measurement using conventional hardware.[10] These NMR supersequences (*i.e.* nested sequences of pulse sequences, extending the original COSY–NOESY combination[11,12]) offer significant time savings as compared to the conventional way of recording NMR spectra due to the fact that several NMR experiments are recorded whilst employing only a single recovery period. They may be distinguished from time-shared experiments[13,14] which collect similar types of spectra in parallel for different nuclei, typically ^{13}C and ^{15}N. Here we describe new NMR supersequences (NOAH-3 BSC and NOAH-4 BSCN) that are specifically designed to allow elucidation of molecular structure from a single measurement in diluted spin systems, typically organic molecules at natural isotopic abundance. We also introduce two new NMR experiments and NOAH modules, the ASAP-COSY and ZZ-HMBC. In addition to the significant time savings provided by the NOAH approach, recording the required spectra in a single measurement benefits also from the fact that all the spectra are recorded under identical conditions and in an identical experimental environment, which may prove especially beneficial when used in automated, computer-assisted structure elucidation (CASE) protocols.[15–17]

In the vast majority of cases the structures of small organic molecules can be established from three basic 2D NMR experiments[18–21] – HSQC,[22] HMBC[23] and COSY.[24] Usually, these spectra are recorded as separate experiments that start with recovery delay, d_1, which is by far the longest event in such experiments. These spectra can be obtained significantly faster by combining these experiments into a single NOAH NMR supersequence that requires only one recovery delay, d_1, thus offering significant time savings over the conventional approach. The NOAH supersequences are constructed by linking individual, specially adapted NMR sequences (NOAH modules) according to the domino principle – the tailored output of one module serves as an input of the following module (Fig. 1).[10] For instance, the HSQC, HMBC and COSY modules can be linked into a NOAH-3 supersequence called SBC (Fig. 1a).

Unfortunately the SBC type supersequences suffer from some drawbacks. Most notably, the bulk magnetization, M following the HMBC module is left in the XY plane and is subjected to transverse relaxation (T_2) effects. Generally the T_2 relaxation can be quite different for individual peaks across the ^{1}H spectra of molecule(s) of interest. This produces differential attenuation of the cross peaks in the 2D COSY spectra. The effect is exacerbated in the NOAH-3 SBC experiment and in

[a] *Bruker UK Ltd., Banner Lane, Coventry, CV4 9GH, UK. E-mail: eriks.kupce@bruker.com*
[b] *Department of Chemistry, University of Oxford, Chemistry Research Laboratory, Mansfield Road, Oxford, OX1 3TA, UK. E-mail: tim.claridge@chem.ox.ac.uk*

† Electronic supplementary information (ESI) available: Details of ASAP-COSY; details of NOAH-4 BSCN; CMCse CASE details and reports for gibberellic acid and 1,2:3,4-*O*-isopropylidene-α-D-galactopyranose; NOAH pulse programmes (PDF). See DOI: 10.1039/c8cc03296c

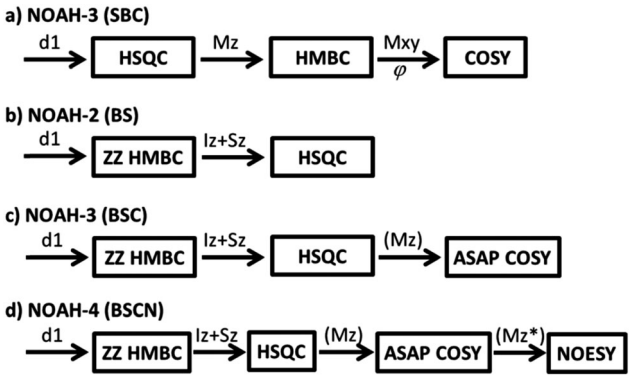

Fig. 1 A modular representation of the NOAH supersequences (a) NOAH-3 SBC, (b) NOAH-2 BS, (c) NOAH-3 BSC and (d) NOAH-4 BSCN; one letter abbreviations are used for HSQC (S), HMBC (B), COSY (C) and NOESY (N) modules. The stored magnetization of the preceding module is used as input magnetization of the following module and is shown above the arrows, the inherited phase, φ is shown below the arrows, M_z* represents t_1-encoded M_z magnetization; partially recovered magnetization is shown in parenthesis.

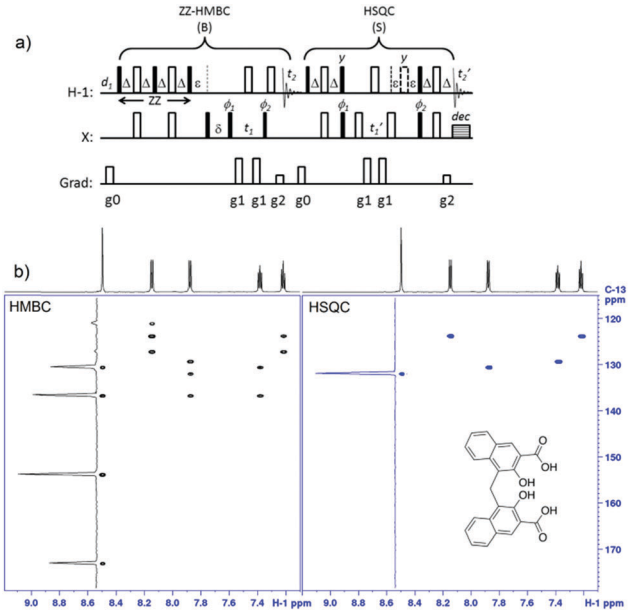

Fig. 2 The NOAH-2 BS experiment for nested acquisition of 2D HMBC and HSQC spectra where X represents a dilute spin, typically ^{13}C, but may also apply to other dilute isotopes such as ^{15}N, ^{29}Si and similar. (a) The pulse sequence showing the ZZ-HMBC (B) and HSQC (S) modules; the filled bars denote 90 degree pulses whereas the hollow rectangles on ^{1}H and X channels denote 180 degree pulses, the dotted rectangle in the HSQC module is an optional 180 degree pulse to provide multiplicity edited HSQC spectra. (b) The spectra of pamoic acid (inset) in DMSO-d_6 recorded with the NOAH-2 BS supersequence. For these data, all ^{13}C 180 degree pulses are adiabatic CA WURST-20 pulses; g0 are spoiler gradients of arbitrary intensities, g1 and g2 are coherence selection gradients applied with a ratio of 2 : 1; delays $\Delta = 0.25/^{1}J_{CH}$, $\varepsilon = 2\Delta$, $\delta = 0.5/^{n}J_{CH}$. The sign of the g2 gradients is inverted in alternate increments for echo–anti–echo encoding; all pulses are applied with phase x unless indicated otherwise; $\phi_1 = x, -x$; $\phi_2 = x, x, -x, -x$; rec = $x, -x, -x, x$.

extreme cases may lead to a loss of information (cross-peaks). A less detrimental, but still significant problem is evolution of the J_{HH} couplings during the HMBC module which tends to enhance long-range H–H correlations seen in COSY.[25] Finally, the J_{HH} evolution during the t_1-evolution period in the HMBC module tends to widen the multiplet structure in the F_1 domain of the COSY spectra.

The drawbacks of the NOAH-3 SBC experiment can be circumvented by swapping around the HSQC and HMBC modules (Fig. 1b). Placing the less sensitive HMBC module before the HSQC module provides also a better balanced experiment. To achieve this we begin the HMBC experiment with an 'external' (preparatory) *ZZ* filter designed to store the $H_z + C_z$ magnetization of the $^{1}J_{CH}$ coupled protons along the $+Z$ axis during most of the HMBC pulse sequence, except for a short period of time during the second half of the t_1-evolution period when this magnetization is aligned with the $-Z$ axis, after which it is returned to $+Z$ by an additional proton inversion pulse (Fig. 2a). Therefore, following the last proton 90 degree pulse at the end of the $^{1}J_{CH}$ *ZZ* filter, the bulk proton magnetization (including long-range $^{n}J_{CH}$ coupled magnetization) is aligned with the X axis while the $H_z + C_z$ magnetization due to the one-bond $^{1}J_{CH}$ couplings is aligned with the $+Z$ axis. This is followed by the usual long-range $^{n}J_{CH}$ ($n > 1$) evolution period, δ, complemented with the conventional $^{1}J_{CH}$ filter for enhanced suppression of the one-bond C–H correlations. We call this module ZZ-HMBC to emphasize that the $H_z + C_z$ magnetization due to the one-bond $^{1}J_{CH}$ couplings at the output of the ZZ-HMBC module has been preserved (that is, aligned with the $+Z$ axis). The NOAH-2 BS supersequence consisting of the ZZ-HMBC (B) and HSQC (S) modules is schematically shown in Fig. 1b and in full in Fig. 2a. The sensitivity comparison and the quality of the 2D HMBC and 2D HSQC spectra recorded using the NOAH-2 BS supersequence are shown in Fig. 2b.

This two-module BS combination forms the basis of the NOAH-3 BSC experiment. While the bulk magnetization is essentially destroyed at the end of the ZZ-HMBC module, some of that magnetization recovers during the HMBC acquisition time, t_2. This partially recovered M_z magnetization is then preserved by the HSQC module and recovers further during the HSQC acquisition period, t_2'. Thus, it can be utilized to record the 2D H–H COSY spectrum. Even though the ^{1}H magnetization is only partially recovered, the observed signal is still by far the strongest when compared to that in the HMBC and HSQC modules that use only 1% of the total ^{1}H magnetization.

As noted above, in the NOAH-3 SBC experiment (Fig. 1a) the intensity of the magnetization used to generate the COSY spectrum is influenced by transverse T_2 relaxation throughout the HMBC module. In the NOAH-3 BSC experiment (Fig. 1c) the partially recovered magnetization (M_z) employed for COSY is also subject to differences in the relaxation rates of individual protons, except in this case it is determined by the longitudinal T_1 relaxation. In order to reduce the variations in crosspeak intensity across the COSY spectrum due to the differential T_1 relaxation, a short spinlock may be applied in analogy with the heteronuclear ASAP (Acceleration by Sharing Adjacent Polarization) experiments (Fig. 3a).[26,27] Accordingly, we call this module ASAP-COSY. Here we use an adiabatic CA WURST-2 mixing sequence for spinlock because of its tolerance to the magnetic

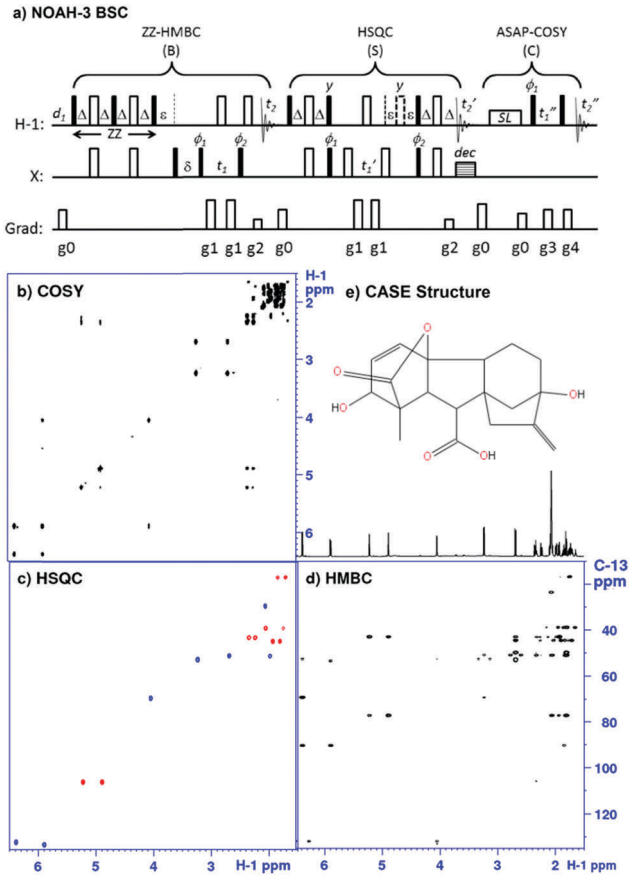

Fig. 3 The NOAH-3 BSC experiment for structure elucidation of small molecules; (a) the NOAH-3 BSC supersequence showing the ZZ-HMBC (B), HSQC (S) and ASAP-COSY (C) modules; SL represents a short spinlock of 40–60 ms, gradients g3 : g4 = 1 : 1; phase rec″ = *x*, −*x*; for other experimental details see caption to Fig. 2; (b–d) the NOAH-3 BSC spectra recorded on a 700 MHz (^{1}H) Bruker AVANCE III NMR spectrometer equipped with the TCI cryoprobe. The data matrix size was 2k × 1536 data points (512 t_1 points per module) recorded with one scan per increment, recovery delay d_1 = 1.5 s; (b) 2D ^{1}H COSY, (c) multiplicity-edited 2D ^{1}H–^{13}C HSQC and (d) 2D ^{1}H–^{13}C HMBC spectra all recorded in the same experiment; the sample is 10 mg of gibberellic acid in 500 µl of acetone-d_6; (e) the highest ranked structure of the molecule generated by the Bruker CMCse structure elucidation software based on spectra (b–d). A detailed CMCse report is provided in the ESI.†

and RF field inhomogeneities and mis-calibration, and due to its clean mixing performance,[28,29] but other mixing sequences can also be used.

This mixing has two important consequences. Firstly, it reduces the peak intensity differences due to the variation of the T_1 relaxation rates leading to more uniform cross-peak intensities in the COSY spectra (see discussions in ESI† and Fig. S1). Secondly, besides the intensity balancing effect the short spinlock period also dramatically reduces the fast pulsing artifacts (Fig. S2 and S3, ESI†). This rather surprising effect has been observed also in heteronuclear ASAP experiments.[26,27]

The ASAP-COSY module is simply appended to the NOAH-2 BS experiment to yield the NOAH-3 BSC supersequence as shown in detail in Fig. 3a. This new supersequence provides high quality 2D ^{1}H–^{13}C HMBC, multiplicity-edited ^{1}H–^{13}C HSQC and ^{1}H–^{1}H COSY spectra (see Fig. 3b–d), in a considerably shorter period of time (*ca.* 20 min) as compared to the conventional way of recording the same spectra (*ca.* 46 min), that can be used for routine structure elucidation of small organic molecules. Here we provide two such examples employing computer-assisted structure elucidation with the CMCse software package.[30,31] Minor NOAH data preparation steps that are required for the CMCse analysis are described in the ESI,† but no changes to the usual workflow for structure elucidation were necessary.

In the first example, the structure of gibberellic acid is solved correctly from the NOAH-3 BSC data (Fig. 3e). The second example (Fig. 4) shows the structure of 1,2:3,4-*O*-isopropylidene-α-D-galactopyranose determined correctly from the spectra recorded using the NOAH-4 BSCN supersequence that is obtained by appending the NOESY module to the NOAH-3 BSC supersequence (Fig. 1d, and see ESI† and Fig. S4). The NOESY module provides additional, stereospecific spatial information about the orientation of the four Me-groups in the molecule that is not available from other data *e.g.* the chemical shifts or $^3J_{HH}$ couplings.

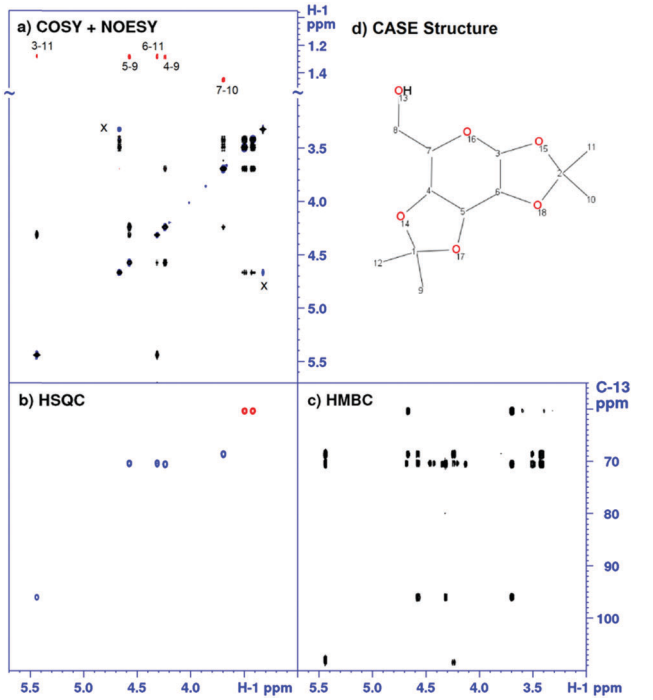

Fig. 4 NOAH-4 BSCN spectra recorded at 700 MHz (^{1}H) on a Bruker AVANCE III NMR spectrometer equipped with the TCI cryoprobe. The data matrix size was 2k × 2k data points (512 t_1 points per module) recorded with one scan per increment, recovery delay d_1 = 1.5 s, the pulse sequence and other experimental details are provided in the ESI;† the sample is 10 mg of 1,2:3,4-*O*-isopropylidene-α-D-galactopyranose in 500 µl of DMSO-d_6; (a) 2D overlaid COSY (magnitude, in black) and 2D NOESY (positive peaks in blue and negative peaks in red) spectra; the positive (blue) peaks marked by '*x*' are due to exchange between the CH_2OH group and H_2O in the sample; the negative (red) NOESY peaks indicating spatial proximity between protons in positions 3, 6 and 11; 4, 5 and 9; 7 and 10 are consistent with the structure of the molecule (CMCse numbering); (b) multiplicity-edited 2D ^{1}H–^{13}C HSQC and (c) 2D ^{1}H–^{13}C HMBC spectra recorded in the same experiment; (d) the highest ranked structure of the molecule generated by the CMCse software based on spectra (a–c).

The NOAH-4 BSCN experiment was recorded in just 26 minutes as compared to *ca.* 70 minutes required to record the data of similar resolution with the conventional pulse sequences.

To conclude, we have presented two new NOAH supersequences optimized for structure elucidation of small organic molecules from a single measurement. The new supersequences are based on two new NOAH modules – ASAP-COSY and ZZ-HMBC. These two modules represent new pulse sequences that can be used also as standalone experiments. It should be noted that the ASAP technique demonstrated here for the COSY module may also be applicable to other homonuclear 2D modules, such as DQF COSY and NOESY. Most importantly, we have demonstrated the practical applications of new NOAH experiments for routine and fast structure elucidation of molecules at natural isotopic abundance.

We would like to thank Dr Till Kühn and Dr Pavel Kessler for help with the CMCse software. All new pulse programmes discussed in this work are provided in the ESI† and will also be available from the Bruker online User Library or alternatively by request from the authors.

Conflicts of interest

There are no conflicts to declare.

Notes and references

1 H. Kovacs, D. Moskau and M. Spraul, *Prog. Nucl. Magn. Reson. Spectrosc.*, 2005, **46**, 131–155.
2 M. Mobli and J. C. Hoch, *Prog. Nucl. Magn. Reson. Spectrosc.*, 2014, **83**, 21–41.
3 Ē. Kupče, in *Modern NMR Methodology*, ed. H. Heise and S. Matthews, Springer, Berlin, 2013, ch. 226, vol. 335, pp. 71–96.
4 H. Kovacs and Ē. Kupče, *Magn. Reson. Chem.*, 2016, **54**, 544–560.
5 Ē. Kupče and R. Freeman, *J. Am. Chem. Soc.*, 2008, **130**, 10788–10792.
6 Ē. Kupče and R. Freeman, *J. Magn. Reson.*, 2010, **206**, 147–153.
7 A. Bax, R. Freeman and S. P. Kempsell, *J. Am. Chem. Soc.*, 1980, **102**, 4849–4851.
8 A. Bax, R. Freeman and S. P. Kempsell, *J. Magn. Reson.*, 1980, **41**, 349–353.
9 A. Bax, R. Freeman, T. A. Frenkiel and M. H. Levitt, *J. Magn. Reson.*, 1981, **43**, 478–483.
10 Ē. Kupče and T. D. W. Claridge, *Angew. Chem., Int. Ed.*, 2017, **56**, 11779–11783.
11 A. Z. Gurevich, I. L. Barsukov, A. S. Arseniev and V. F. Bystrov, *J. Magn. Reson.*, 1984, **56**, 471–478.
12 C. A. G. Haasnoot, F. J. M. V. d. Ven and C. W. Hilbers, *J. Magn. Reson.*, 1984, **56**, 343–349.
13 P. Nolis, M. Pérez-Trujillo and T. Parella, *Angew. Chem., Int. Ed.*, 2007, **46**, 7495–7497.
14 T. Parella and P. Nolis, *Concepts Magn. Reson., Part A*, 2010, **36**, 1–23.
15 M. E. Elyashberg, A. J. Williams and G. E. Martin, *Prog. Nucl. Magn. Reson. Spectrosc.*, 2008, **53**, 1–104.
16 J.-M. Nuzillard, *eMagRes*, 2014, **3**, 287–294.
17 A. V. Buevich and M. E. Elyashberg, *J. Nat. Prod.*, 2016, **79**, 3105–3116.
18 T. D. W. Claridge, *High-Resolution NMR Techniques in Organic Chemistry*, Elsevier, Boston, 3rd edn, 2016.
19 W. F. Reynolds and R. G. Enriquez, *J. Nat. Prod.*, 2002, **65**, 221–244.
20 R. C. Breton and W. F. Reynolds, *Nat. Prod. Rep.*, 2013, **30**, 501–524.
21 M. Elyashberg, *TrAC, Trends Anal. Chem.*, 2015, **69**, 88–97.
22 G. Bodenhausen and D. J. Ruben, *Chem. Phys. Lett.*, 1980, **69**, 185–189.
23 A. Bax and M. F. Summers, *J. Am. Chem. Soc.*, 1986, **108**, 2093–2094.
24 J. Jeener, Ampère International Summer School, Basko Polje, 1971.
25 A. Bax and R. Freeman, *J. Magn. Reson.*, 1981, **44**, 542–561.
26 Ē. Kupče and R. Freeman, *Magn. Reson. Chem.*, 2007, **45**, 2–4.
27 D. Schulze-Sünninghausen, J. Becker and B. Luy, *J. Am. Chem. Soc.*, 2014, **136**, 1242–1245.
28 Ē. Kupče, P. Schmidt, M. Rance and G. Wagner, *J. Magn. Reson.*, 1998, **135**, 361–367.
29 Ē. Kupče and W. Hiller, *Magn. Reson. Chem.*, 2001, **39**, 231–235.
30 Complete Molecular Confidence-Structure Elucidation, Bruker BioSpin GmbH, 2018.
31 P. Kessler and M. Godejohann, *Magn. Reson. Chem.*, 2018, **56**, 480–492.

