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BANGO SEA XLOC/HMBC–H2OBC: Complete heteronuclear correlation within minutes from *one* NMR pulse sequence

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Novel NMR experiments, BANGO SEA XLOC–H2OBC or BANGO HMBC–H2OBC, can deliver complete heteronuclear correlation maps on a time scale of minutes for small molecules. By way of example, it is demonstrated that all intra- and inter-residue ¹H and ¹³C correlations and assignments of a trisaccharide are obtained in 20 or 5 minutes of instrument time without or with 25% NUS, respectively.

Extracting maximum information in minimum time has always been an objective in NMR spectroscopy and many approaches to that effect have been described over the years. It can e.g. be by extracting different spectra out of the same data set, different types of time-shared acquisitions, or sequential or interleaved acquisition where different pools of magnetization are exploited.

Although not true for all experiments of this type¹ there often are compromises on sensitivity or resolution when different experiments are to be accommodated within the same overall pulse sequence. The individual experiments typically have different sensitivities and it usually does not matter if those of highest sensitivity are compromised on that parameter, but it is critical that those of lowest sensitivity are not compromised significantly. Otherwise, often there is no advantage over separate acquisition of the experiments in question, unless sensitivity is not an issue.

With the starting point of what is needed and available to distinguish heteronuclear two- and three-bond correlations for all multiplicities and coupling patterns this Communication combines relevant experiments into a single pulse sequence with the aim to extract as much information as possible from it in the shortest possible time.

The main building blocks are SEA XLOC² and H2BC.³ The former relies on ⁿJ_{CH} for coherence transfer, and the number of intervening

bonds is indicated by which of the associated double-quantum (2Q) and zero-quantum (ZQ) multiplets is the wider in spectra in the usual format of ¹³C frequencies in F₁ and ¹H frequencies in F₂. Due to the typically negative ²J_{CH} coupling constants being passive in three-bond correlations these multiplets are narrower in the 2Q subspectrum, whilst the ZQ multiplets are the narrower ones for two-bond correlations.

Where ²J_{CH} vanishes there are no peaks in SEA XLOC spectra, but fortunately the corresponding correlation peaks can be seen in H2BC spectra provided the associated ³J_{HH} does not vanish. That is the basis for the recommendation to record both spectra to establish as many long-range correlations as possible along with the distinction between two- and three-bond peaks.

SEA XLOC and H2BC rely on different pools of magnetization, as the former uses ¹H magnetization *not* attached to ¹³C, in contrast to the latter which uses magnetization attached to ¹³C. Thus if these two pools of magnetization are manipulated independently, SEA XLOC and H2BC spectra can be recorded sequentially within the same overall pulse sequence. Related sequential recording of multiple experiments has been the subject of recent work by Kupče and Claridge.⁴ In comparison, our pulse sequences offer more efficient initial discrimination between the two pools of magnetization, less inversion pulses, and time-saving from not having HSQC and COSY as separate additional modules in the overall pulse sequence.

To perform the independent manipulations in the two pools of magnetization, the BANGO⁵ pulse sequence element is useful. The rotations by angles β^I and β^{IS} for I spins not attached and attached to an S spin, respectively, are delivered by BANGO in its general outline:

$$\left(\frac{\beta^I - \beta^{IS}}{2}\right)_x^I - \frac{1}{2J_{IS}} - (\pi)_x^{I,S} - \frac{1}{2J_{IS}} - \left(\pi + \frac{\beta^I + \beta^{IS}}{2}\right)_x^I$$

where the superscripts on the parentheses indicate on which spin channel the pulses are applied. BANGO can be visualized and described fully by the vector model. Well-known pulse sequence elements like BIRD⁶ and TANGO⁷ in all their variations are special

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cases of BANGO, as can be verified by insertion of appropriate rotation angles β^I and β^{IS} .

Insertion of $\beta^I = -\pi/2$ and $\beta^{IS} = -\pi$ in the BANGO element above leads to:

$$\left(\frac{\pi}{4}\right)_x^I - \frac{1}{2J_{IS}} - \pi_x^{IS} - \frac{1}{2J_{IS}} - \left(\frac{\pi}{4}\right)_x^I$$

a pulse sequence that was also mentioned in the TANGO paper. For the current application, spin I represents ^1H and S represents ^{13}C . As specified at the outset the BANGO element acts as a $\pi/2$ pulse for ^1H magnetization *not* attached to ^{13}C and as π pulse for ^1H magnetization attached to ^{13}C . Thus the element is ideal as replacement for the ^1H $\pi/2$ excitation pulse in SEA XLOC for two reasons: 1) only the SEA XLOC pool of magnetization gets excited and 2) inversion of ^1H magnetization attached to ^{13}C makes the ^1H π pulse in SEA XLOC return that magnetization to the positive z axis to take advantage of T_1 relaxation and be ready for the succeeding H2BC part of the combined pulse sequence that is applied without modification compared to its stand-alone version.

Without extending the pulse sequence in any way, also a one-bond ^1H - ^{13}C correlation spectrum with the appearance of HSQC can

be extracted from the data in the H2BC part, i.e. in the style of 2BOB.^{1b} Extracting the one-bond spectrum in this way in no way compromises the sensitivity of the other experiments.

The fastest way to obtain both the long-range and one-bond information is to combine SEA XLOC with H2OBC^{1b} where the latter contains HSQC-type and H2BC peaks appearing in the same spectrum with different phases. That pulse sequence is outlined in Fig. 1 in an up/down version in the H2OBC part, i.e. with odd and even multiplicities of the ^{13}C involved appearing with opposite phases in that spectrum. The up/down pattern is opposite in the one-bond and two-bond subspectra, which allows for easy visual distinction. Identical up/down patterns in the two subspectra can be obtained by a slightly shorter pulse sequence outlined in ESI. The shorter sequences are expected to have marginally higher sensitivity due to the shorter lengths and having one $\pi(\text{C})$ pulse less.

A J deviating from $(J_{\text{max}} + J_{\text{min}})/2$ in BANGO potentially reduces the sensitivity in the H2OBC part, but there are no spectral artifacts as a result of such a deviation and some of that sensitivity loss can be regained by T_1 relaxation after the last $\pi(\text{H})$ pulse of the preceding SEA XLOC part.

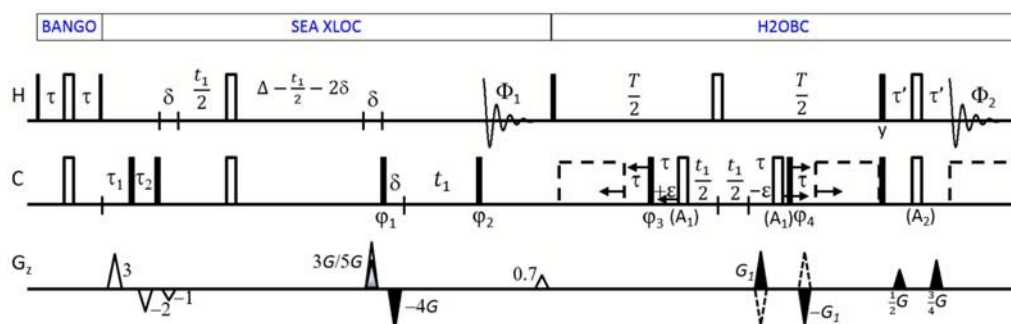
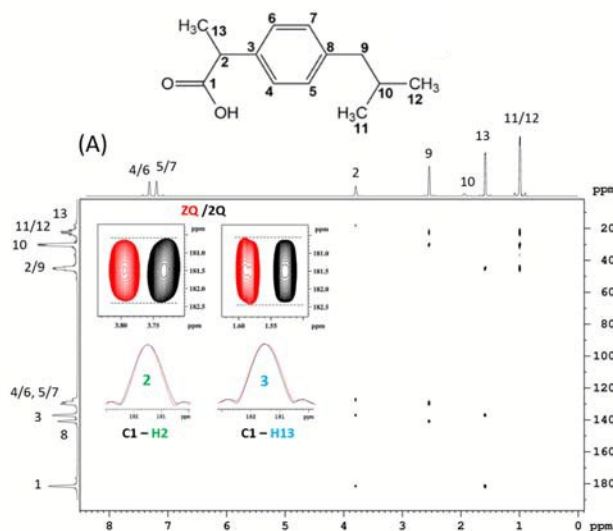


Figure 1. BANGO SEA XLOC-H2OBC pulse sequence. Narrow filled, wider filled and open bars refer to $\pi/4$, $\pi/2$ and π pulses, respectively. A_1 and A_2 are adiabatic inversion, CAWURST-20 pulses (0.5 ms, 300 ppm, low-to-high frequency sweep). The BANGO delay τ is set to $(J_{\text{max}} + J_{\text{min}})^{-1}$. A 2nd order low-pass J filter is employed with $\tau_1 = 0.5[J_{\text{min}} + 0.146(J_{\text{max}} - J_{\text{min}})]^{-1}$ and $\tau_2 = 0.5[J_{\text{max}} - 0.146(J_{\text{max}} - J_{\text{min}})]^{-1}$. $\tau' = \tau - t(A_2)/2$. $\epsilon = t(\pi^4)/2$. The delays Δ and T are for evolution under heteronuclear long-range and homonuclear proton-proton couplings, respectively. δ a gradient delay. The amplitude of the purging gradients (open triangle) can be set an order of magnitude lower than the amplitude of the other ones selecting coherence transfer echo and antiecho. $\phi_1/\phi_3 = \{x, -x, -x, x\}$, $\phi_2/\phi_4 = \{x, x, -x, -x\}$, and receiver phases $\Phi_1/\Phi_2 = \{x, -x\}$. Before standard processing the obtained combined data set is separated into two blocks, corresponding to ZQ/2Q SEA XLOC and H2OBC data, using the Bruker au-program *splitx*. Then the data block of SEA XLOC is divided further with the au-program *split* to obtain ZQ and 2Q data separately. Finally, the ZQ and 2Q SEA XLOC and H2OBC data are processed as in their standalone experiments. The pulse sequence code for Bruker spectrometers can be found in Supplementary Information.

A demonstration of the BANGO SEA XLOC-H2OBC experiment applied to ibuprofen is presented in Figs. 2 and 3. It should be recognized that the information obtained from such 42 minutes of instrument time on BANGO SEA XLOC-H2OBC corresponds to what is accomplished from the four experiments SEA XLOC, H2BC, HSQC and COSY. If sensitivity is not an issue the time limiting factor is the need to have a digital resolution in the indirect dimension of 70-80 Hz or better in the SEA XLOC spectrum. Compared to running SEA XLOC and H2OBC separately with identical parameters 41% time was saved. If the alternative were to also run HSQC and COSY separately the time saving would, of course, be even bigger.



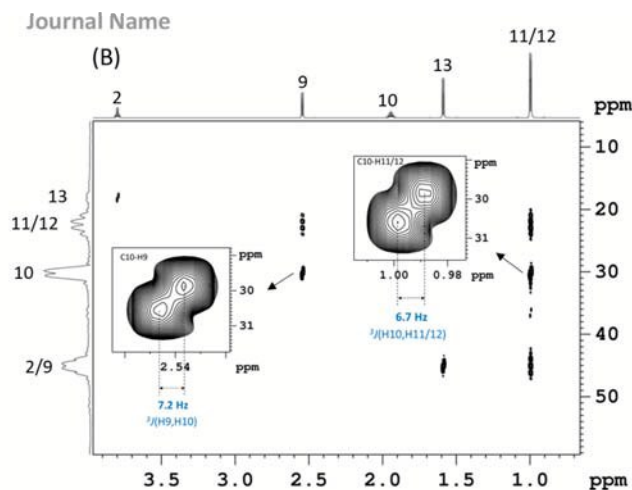


Figure 2. (A) 700 MHz absolute-value SEA XLOC ZQ spectrum of ibuprofen (0.5 M in CDCl_3) recorded on a Bruker Avance NEO spectrometer equipped with a TCI z-gradient prodigy probe using the pulse sequence in Fig. 1. Two- and three-bond correlations of C1 quaternary shown in the inset together with the corresponding 1D sections demonstrate the difference of multiplet widths of ZQC and ZQC peaks, thus identifying the number of intervening bonds as two and three, respectively. The contour plots of ZQC peaks (black) are displaced horizontally with respect to ZQC peaks (red) for better visualization. (B) Protonated carbon region of spectrum A. The E.COSY pattern of two-bond correlation peaks of C10-H9 and C10-H11/H12 shown as insets identifies the pertinent $^3J_{\text{HH}}$ as positive and a digital resolution resolving $^1J_{\text{CH}}$ would allow accurate measurement of $^3J_{\text{HH}}$. The spectrum was acquired with the parameters: $\Delta = 83$ ms, $T = 23$ ms, $^1J_{\text{min}} = 125$ Hz and $^1J_{\text{max}} = 165$ Hz, spectral widths of 8.4 ppm (^1H) and 190.0 ppm (^{13}C), relaxation delay 1.7 s, 512 points in t_1 giving a digital resolution of 65 Hz/point with 1 scan per increment and 2048 data points in t_2 .

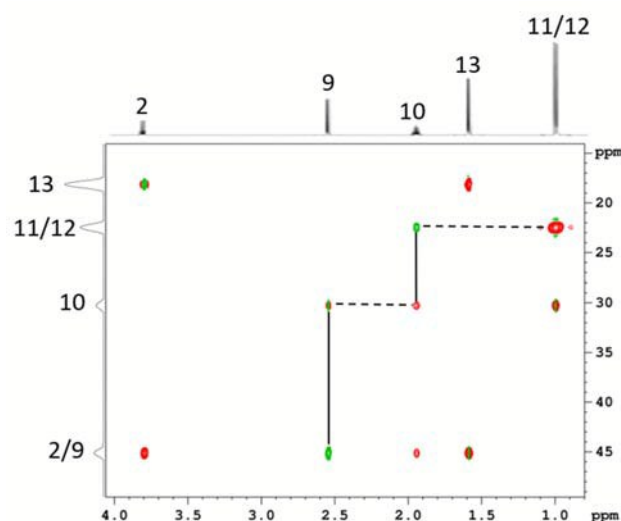


Figure 3. Excerpt of H2OBC spectrum of ibuprofen acquired with the experiment in Fig. 1, illustrating the 'molecular walk' along the C9-C10-C11/C12 carbon chain. Horizontal lines connect neighboring ^1H whilst vertical lines connect neighboring ^{13}C .

If H2OBC instead is combined with HMBC⁸ which does not require that good resolution the experiment HMBC-H2OBC can on ibuprofen be performed in about 11 minutes, as demonstrated in Fig. 4. The excitation sequence replacing the first ^1H $\pi/2$ excitation of HMBC (Fig. S1) is the same BANGO version as above. Thus 11 minutes of instrument time delivers information corresponding to the spectra HMBC, H2BC, HSQC and COSY. Compared to running

HMBC and H2OBC separately with identical parameters 42% time was saved.

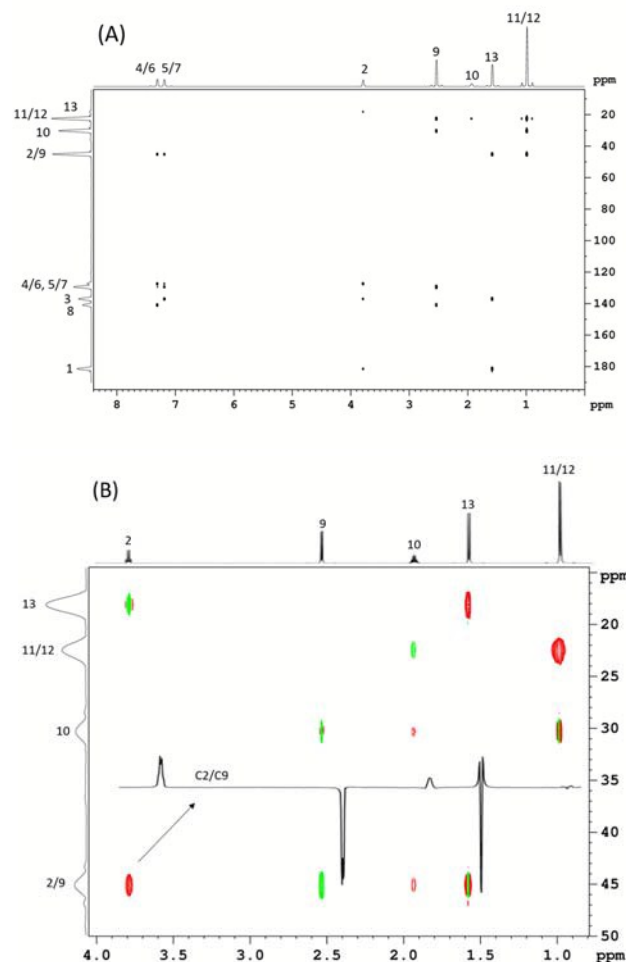


Figure 4. HMBC (A) and H2OBC (B) spectra of ibuprofen recorded in 11 minutes using the BANGO HMBC-H2OBC experiment. The spectra were acquired using the parameters as given in Fig. 2 except for performing only 1 scan per increment and only 128 points in the t_1 period giving a digital resolution of 260 Hz/point. The inset in (B) illustrates the odd/even multiplicity up/down feature by way of the one-bond correlations and the F_2 $\pi/2$ phase shift of antiphase two-bond correlations. The scheme of the BANGO HMBC-H2OBC experiment (Fig. S1) and the pulse sequence code for Bruker spectrometers can be found in Supplementary Information.

The sensitivity of the experiments presented and applied to ibuprofen has been compared to the sensitivity of the standalone experiments. On average, there was a 6% and 8% loss for H2OBC and SEA XLOC, respectively, whilst the HMBC spectrum shows a 6% gain (see ESI). These variations have so far not been investigated further, but a small loss was expected from applying BANGO instead of a $\pi/2$ excitation pulse.

If sensitivity allows it, the experiment time of the BANGO HMBC-H2OBC experiment can be further reduced utilizing the non-uniform sampling (NUS)^{9,10} strategy for recording the F_1 frequency dimension. An example for such a quick experiment on a trisaccharide is shown in Fig. 5. The resulting H2OBC and HMBC

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spectra acquired in 5 minutes allowed the unambiguous assignment of all ^1H and ^{13}C resonances following the 'molecular walk' as shown below in the H2OBC spectrum (left). Moreover, with detection of the respective interglycosidic long-range correlations (labeled with dotted lines in the structure) in the HMBC spectrum (right) the sequential order of sugar residues could be established too. Compared to running HMBC and H2OBC separately with identical parameters 45% time was saved.

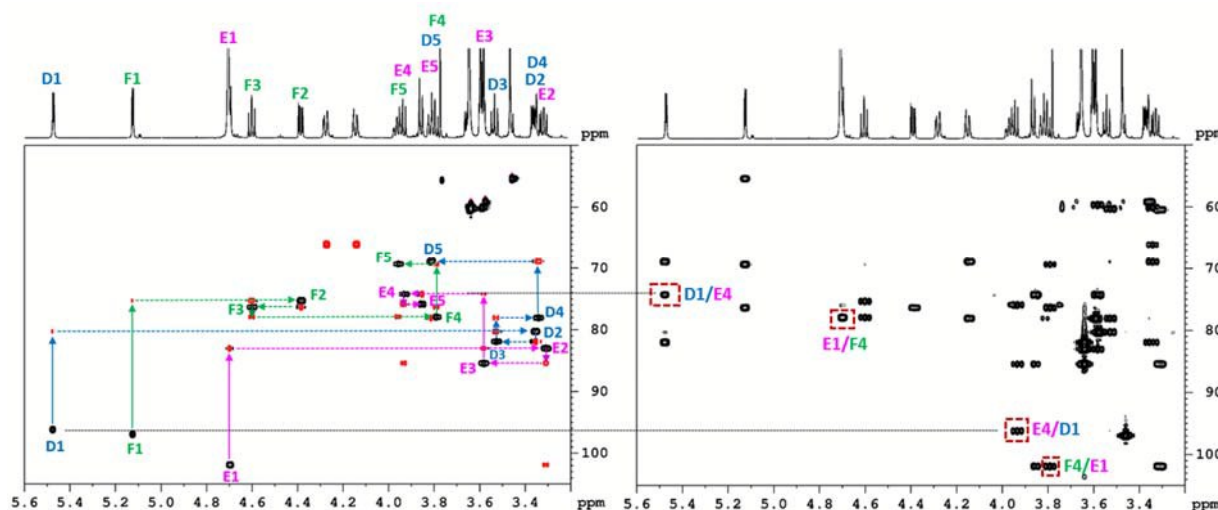
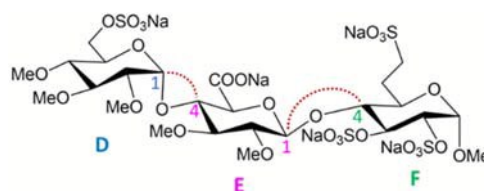


Figure 5. Excerpts of H2OBC (left) and HMBC spectra of a trisaccharide (120 mg in CDCl_3 , structure shown above the spectra) recorded in 5 minutes using the BANGO HMBC-H2OBC experiment with 25 % NUS. The molecular walks of D, E and F residues are labeled by colored dotted lines in the H2OBC spectrum. Peaks confirming the sequential connectivities of D-E and E-F residues are indicated and assigned in the HMBC spectrum. For recording the NUS spectra, a sine-weighted Poisson-gap sampling scheme using the default seed value of the random number generator implemented within TopSpin 4.05 was applied. The spectra were acquired with the parameters: $\Delta = 83$ ms, $T = 23$ ms, $J_{\text{min}} = 125$ Hz, $J_{\text{max}} = 165$ Hz, spectral widths of 5.1 ppm (^1H) and 190.0 ppm (^{13}C), relaxation delay 1.7 s, using 64 NUS points in t_1 with 1 scan per increment and 1024 data points in t_2 . Non-uniformly sampled spectra were reconstructed with compressed sensing (CS) approach implemented in TopSpin.

In conclusion, we have introduced new pulse sequences able to trace out all ^1H and ^{13}C correlations and assignments on a time scale of minutes on small molecules. The pulse sequence element BANGO is used for initial excitation efficiently discriminating between pools of magnetization to be exploited in the first and second part of the overall pulse sequence.

Conflicts of interest

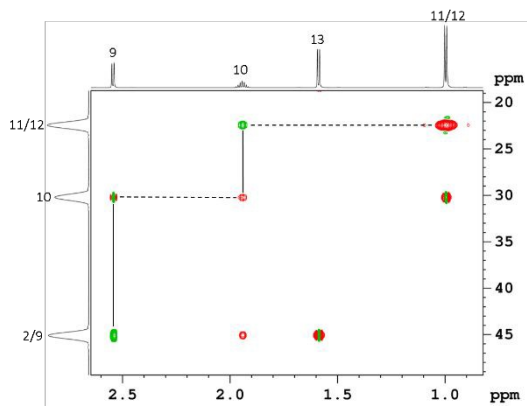
There are no conflicts to declare.

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Novel NMR experiments, BANGO SEA XLOC–H2OBC or BANGO HMBC–H2OBC, deliver complete heteronuclear correlations and assignments on a time scale of minutes on small molecules.