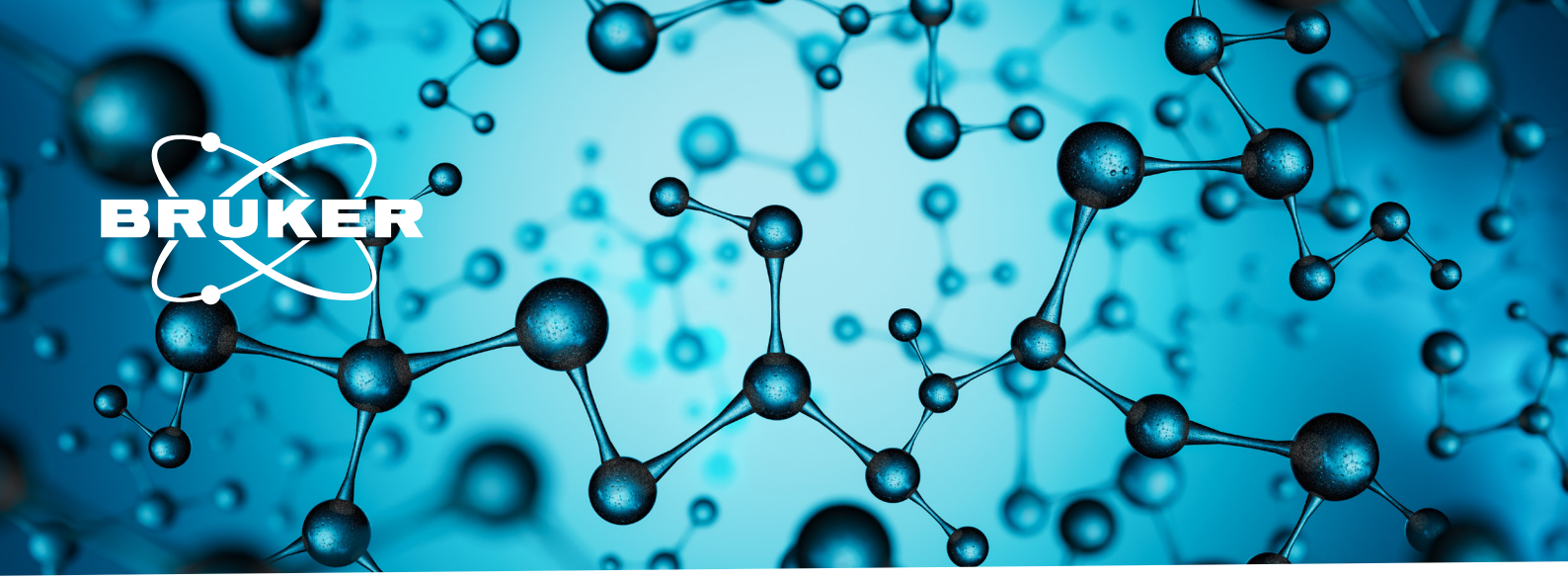

FOURIER 80

Analysis of Brucine on Fourier 80 Benchtop Spectrometer Using ^{1}H and ^{13}C NMR Spectroscopy

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Innovation with Integrity

Brucine is a naturally occurring alkaloid that contributes to the toxic effects of Nux-vomica seeds found in trees native to southern Asia[1]. The chemical structure of brucine, shown in **Figure 1**, contains several interesting functional groups, including a benzene ring, several aliphatic ring structures, and heteroatoms such as nitrogen and oxygen. This array of features gives rise to an NMR spectrum rich in structural content. The Fourier 80 MHz benchtop NMR readily tackles investigations of substances such as brucine, as shown in the following Applications Note below, through 1D and 2D NMR experiments.

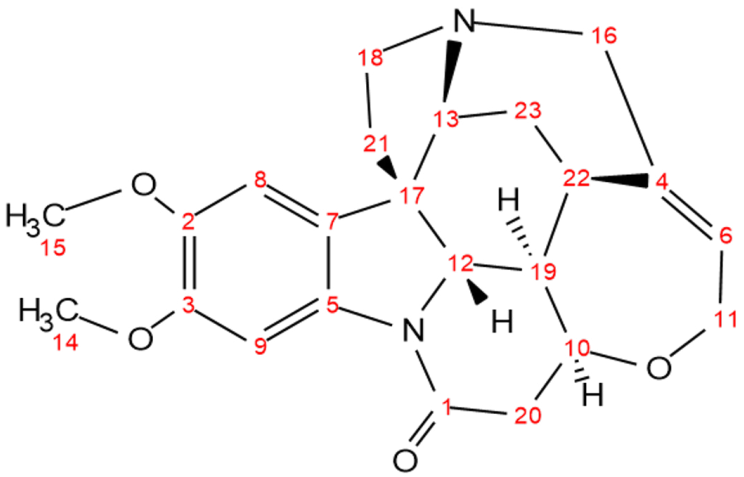

Figure 1 Chemical structure of brucine ($C_{23}H_{26}N_2O_4$).

The ^{1}H NMR spectrum for a 100 mM brucine sample in $CDCl_3$ obtained in 15 seconds with a single scan on a Fourier 80 benchtop spectrometer is shown in **Figure 2**. The spectrum integrates to the expected 26 protons, some with distinct chemical environments, such as the three protons attached to carbons 8, 9, and 6 involved in double bonding.

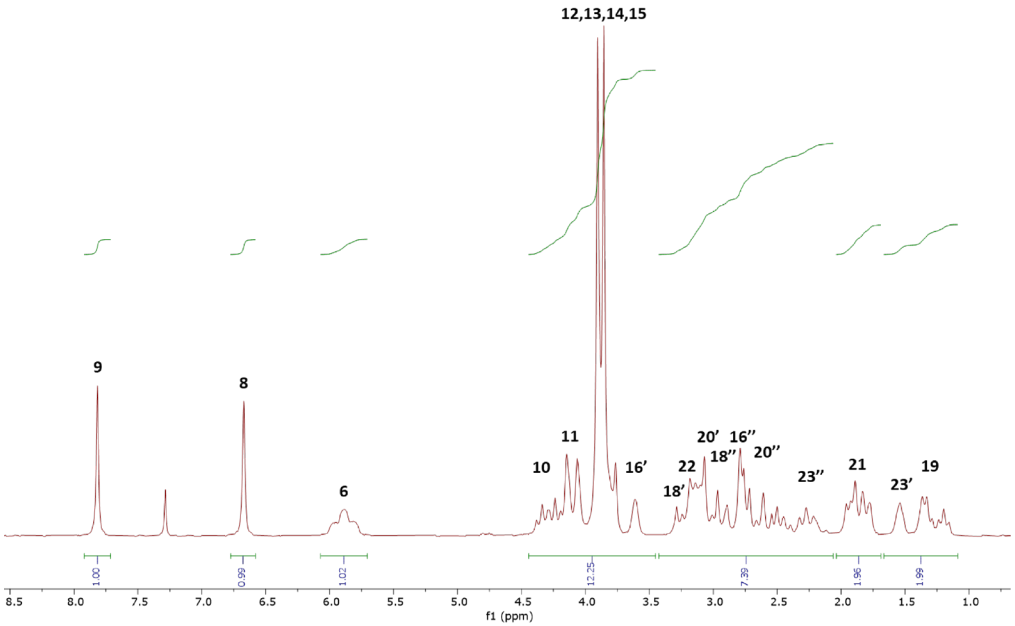

Figure 2 ^{1}H NMR spectrum for 100 mM brucine in $CDCl_3$ acquired in a single scan for an overall experimental time of 15 seconds.

A COSY experiment can be used to correlate nuclei to other nearby nuclei through their chemical bonds. An example ^{1}H COSY experiment is shown in **Figure 3** for 250 mM brucine in $CDCl_3$. Using gradients, this experiment takes 13 minutes to perform with 1 scan per t1 increment. Correlations are observed between peaks assigned in **Figure 2**, such as those between the protons attached to carbons 8 and 9 (blue), 12-14 and 9 (green), 12-14 and 8 (orange), 11 and 6 (violet), 19 and 10 (pink), and 19 and 12-14 (light orange).

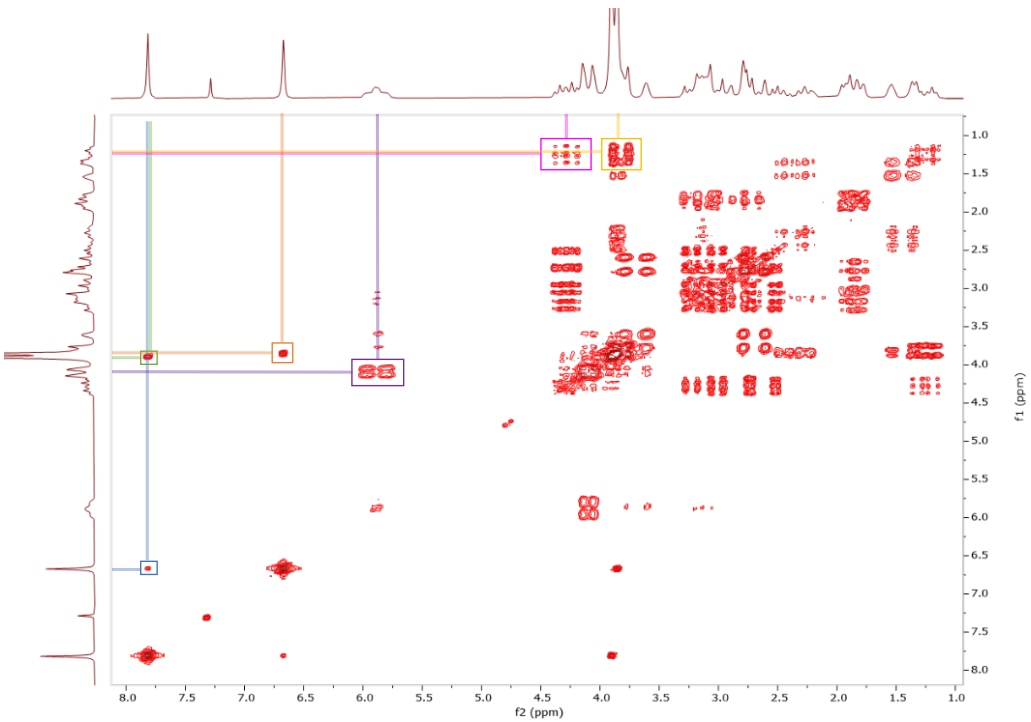

Figure 3 ^{1}H COSY spectrum for 250 mM brucine in $CDCl_3$ acquired in 13 minutes with a single scan per t1 increment.

A ^{13}C spectrum acquired on 1 M brucine in $CDCl_3$ allows for the identification of each of the 23 carbon sites in the molecule, as shown in **Figure 4**. This spectrum is acquired with power-gated ^{1}H decoupling and a 45 degree flip angle with 256 scans at 3 seconds per scan, resulting in a 13 minute experimental time.

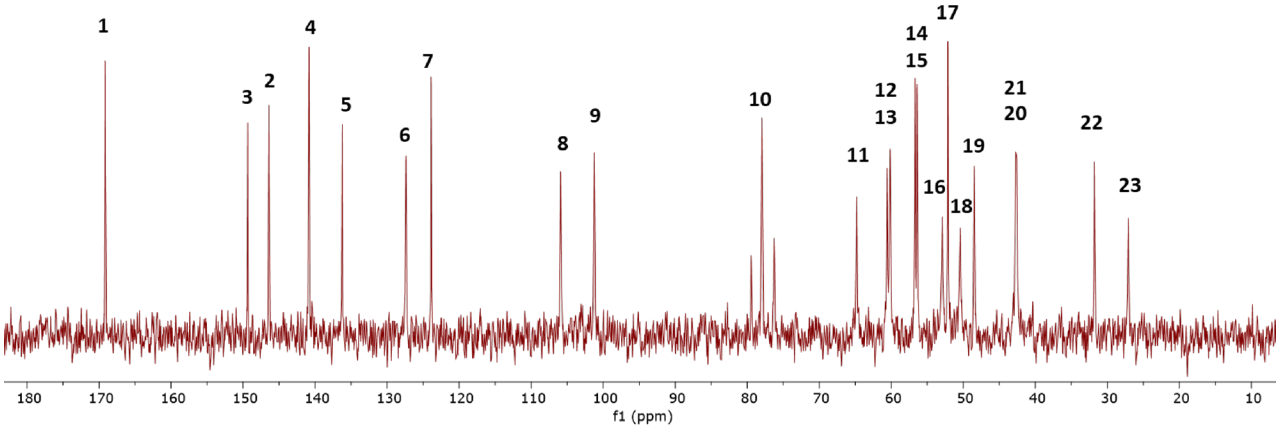

Figure 4 ^{13}C NMR spectrum for 1 M brucine in $CDCl_3$ acquired in 13 minutes with 256 scans with power-gated ^{1}H decoupling and a 45 degree flip angle.

Lastly, 2D experiments making use of the ^{13}C channel can be performed, such as the multiplicity edited HSQC as well as the HMBC shown in **Figure 5**. In the multiplicity edited HSQC, protons in CH_3 and CH groups (blue contours in **Figure 5, left**) appear in opposite phase to those attached to CH_2 groups (red contours in **Figure 5, left**). Carbons with no directly attached protons will not display any correlations. With 4 scans and 128 t1 increments, this experiment took 11 minutes to complete.

In contrast to the HSQC, which shows correlations between protons directly bound to carbons in the molecule, the HMBC allows for the identification of protons that are involved in long-range coupling with other carbons in the molecule (**Figure 5, right**). For example, the protons attached to carbon 9 exhibit long-range coupling to four different carbons as detected in the HMBC: carbons 7 (**blue**), 5 (**green**), 2 (**pink**), and 3 (**violet**), which are all quaternary carbons in the aromatic ring, shown in **Figure 1**. With 8 scans and 256 t1 increments, this experiment required 37 minutes to complete.

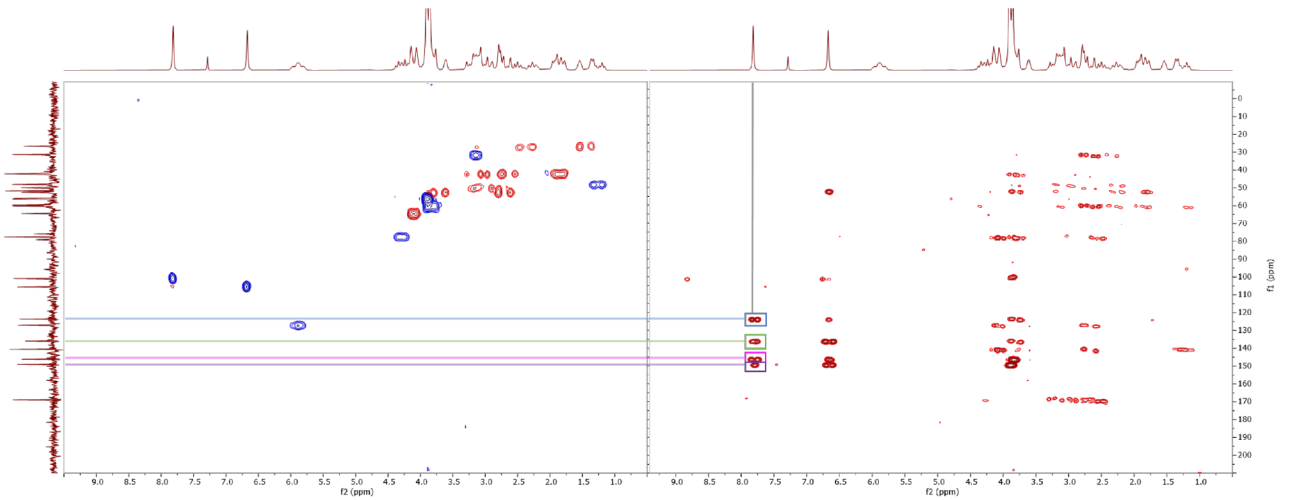

Figure 5 A multiplicity edited HSQC (left) acquired in 11 minutes and an HMBC (right) acquired in 37 minutes for 300 mM brucine in $CDCl_3$.

From the assigned ^{13}C spectrum in **Figure 4**, some peaks are shown to overlap such as carbons 14 and 15 (the methoxy groups) near 56 ppm or carbons 21 and 20 in the aliphatic ring structure, near 42 ppm. This can be disadvantageous when trying to determine the complete chemical structure of an unknown compound. To improve the resolution in the ^{13}C dimension, a greater number of t1 increments may be used. This increases the acquisition time along the indirect dimension, thereby increasing the overall experiment time. For example, the 128 t1 increments in **Figure 5** corresponds to an acquisition time in the indirect dimension of 20 ms. Increasing the t1 increments to 512 corresponds to a longer indirect acquisition time of 70 ms and increases the overall experiment time from 11 minutes to 45 minutes.

One approach to maintaining short overall experiment times while still gaining the additional resolution information achieved by long indirect acquisition times is to implement Non-Uniform Sampling (NUS). The Fourier 80 can readily acquire NUS data, as shown in **Figure 6B**. With 25% sparse sampling, 64 hypercomplex points detected in the indirect dimension, and 512 t1 increments, a multiplicity-edited HSQC can be acquired in 11 minutes with an indirect acquisition time of 70 ms, thus achieving greater resolution in the ^{13}C dimension compared to the non-NUS data (**Figure 6A**) without increasing the overall experiment time.

Despite the increased resolution in the ^{13}C dimension attainable with NUS, some contours in the HSQC are still conjoined or inseparable. In this case, a band-selective HSQC can be advantageous, as shown in **Figures 6C and 6D**. In **Figure 6C**, a band-selective HSQC is acquired from 67 to 47 ppm with 128 t1 increments (yellow region indicated in the ^{13}C projection in **Figure 6A**). Through this experiment, two distinct contours are observed for the two methoxy carbons in the molecule, carbons 14 and 15 in **Figure 1**, near 57 ppm (**red box in Figures 6A, 6B, and 6C**) as well as increased resolution in the contours belonging to carbons 12 and 13 in **Figure 1** (**orange box in Figures 6A, 6B, and 6C**) which reside near nitrogen in the aliphatic rings. Lastly, a band-selective HSQC can be acquired over a narrower range (47 to 37 ppm, green region in the ^{13}C projection in **Figure 6A**) with increased signal averaging of 32 scans and 64 t1 increments, thus allowing for the discrimination of peaks belonging to carbons 21 and 20 (**blue box in Figures 6A, 6B, and 6D**).

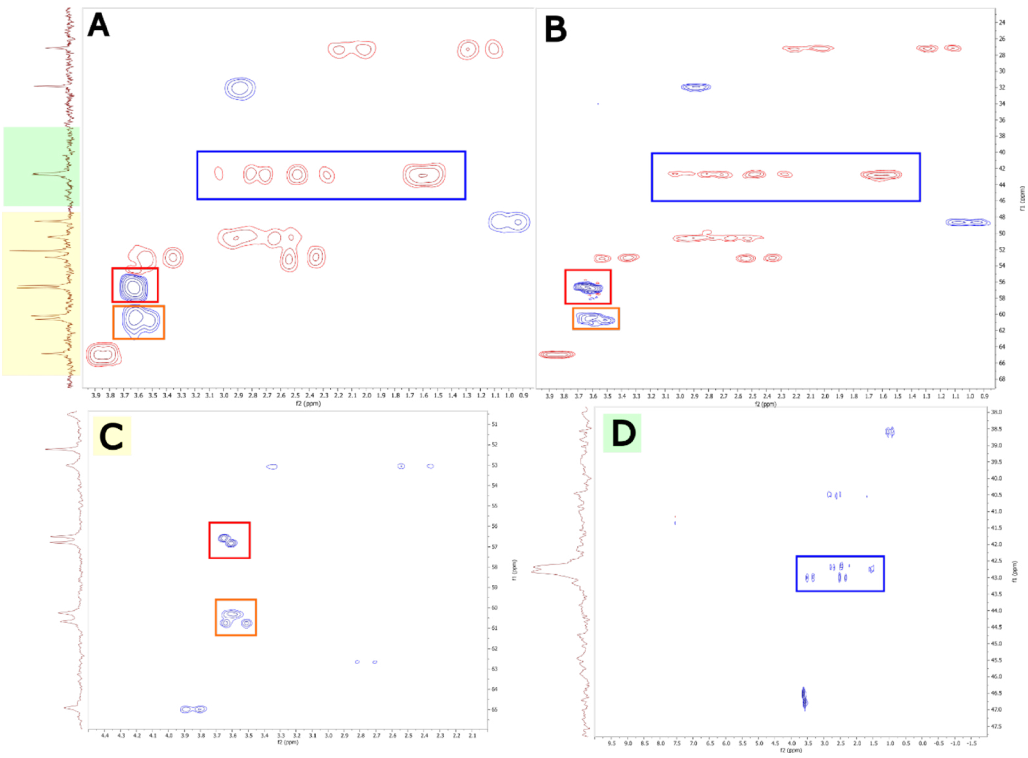

Figure 6 **(A)** Multiplicity edited HSQC acquired in 11 minutes with 128 t1 increments and 4 scans. **(B)** Multiplicity edited HSQC acquired in 11 minutes with 512 t1 increments and 4 scans using NUS. **(C)** Band-selective HSQC from 67 to 47 ppm acquired in 18 minutes with 128 t1 increments and 4 scans. **(D)** Band-selective HSQC acquired from 47 to 37 ppm acquired in an hour and 18 minutes with 64 t1 increments and 64 scans.

As a complement to the COSY spectrum shown in **Figure 3**, a ROESY spectrum can be acquired, as shown in **Figure 7**. In contrast to COSY cross peaks, ROESY cross peaks are not generated by J-coupling through chemical bonds; rather, ROESY cross peaks rely on dipolar coupling, ultimately corresponding to protons that are close to one another in space. ROESY cross peaks will also appear with different phase relative to diagonal peaks, as shown by the **blue contours in Figure 7** relative to the red contours along the diagonal.

As an example of the utility of such information, one can again consider the two methoxy groups (carbons 15 and 14) in **Figure 1**. These methoxy groups can be differentiated in a ROESY spectrum by determining with which benzene ring proton (on carbons 8 and 9) they exhibit a cross peak, as shown in **Figure 7**. These methoxy peaks can be separated from one another by acquiring two 1D Selective ROESY experiments, with each experiment selecting a different proton on the benzene ring. These experiments are simple to set up with an intuitive flow-bar interface in TopSpin.

Once acquired, the resulting spectrum is a horizontal slice through the position of the selected peak on the diagonal of the 2D ROESY spectrum, as shown in **Figure 7.** For example, the higher frequency benzene proton at 7.55 ppm on carbon 9 exhibits a cross peak with the methoxy protons on carbon 14 (**indicated in orange in Figure 7**), whereas the lower frequency benzene proton at 6.40 ppm on carbon 8 exhibits a cross peak with the methoxy protons on carbon 15 (**indicated in green in Figure 7**), with individual methoxy proton resonances observed in each selective 1D spectrum.

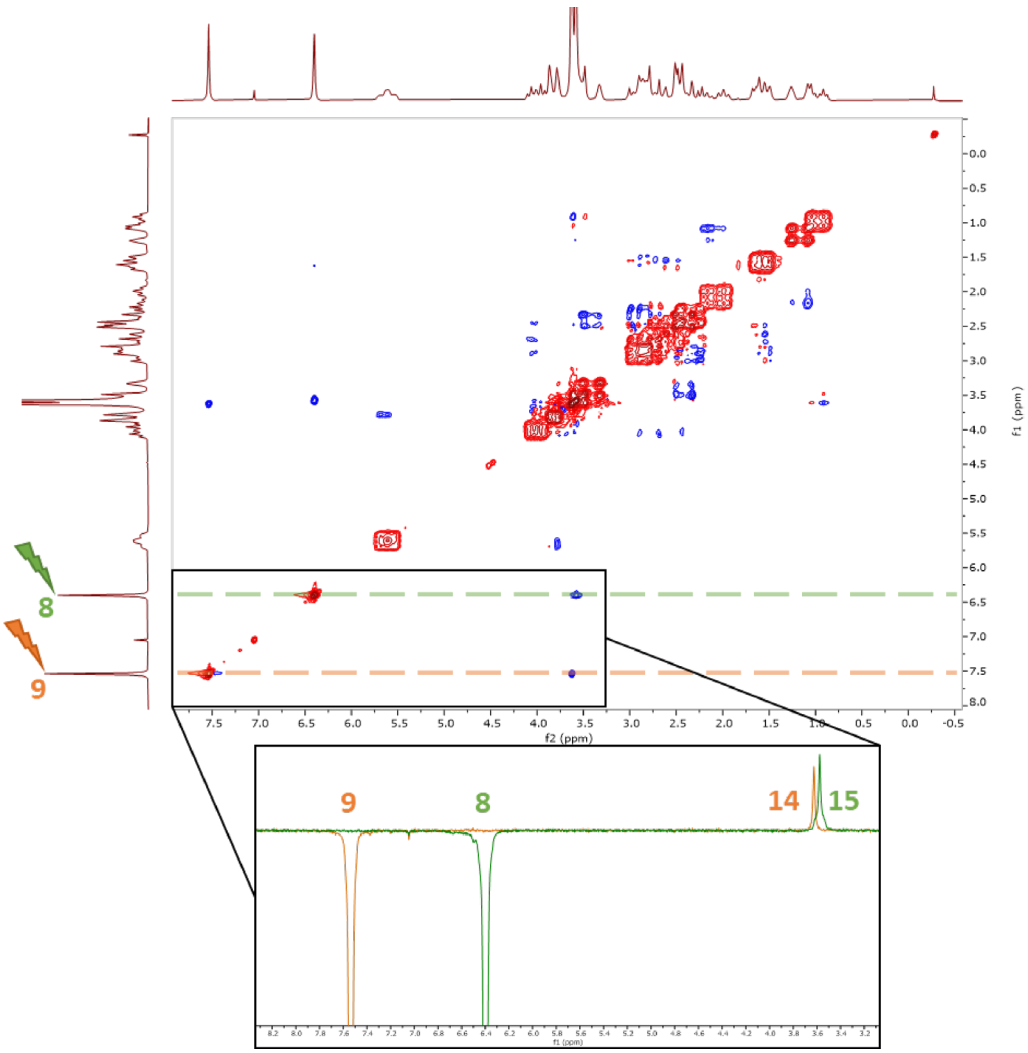

Figure 7 ROESY spectrum of 300 mM brucine at 80 MHz, acquired with 8 scans and 256 t1 increments, for a total experiment time of 1 hour. The inset shows an overlay of two selective 1D ROESY experiments, selecting the peak at 7.55 ppm (**orange**) and 6.40 ppm (**green**) acquired with 8 scans and 2 minutes and 29 seconds experiment time.

In addition to selective ROESY experiments, selective COSY and TOCSY experiments are also possible. Analogous to the 1D Selective ROESY data presented in **Figure 7**, these selective experiments extract a horizontal slice of the full 2D COSY or TOCSY spectrum through the position of the selectively excited peak on the diagonal. In the spectra presented in **Figure 8**, the proton on carbon 6 was selectively irradiated (5.7 ppm, indicated by red lightning bolt in the maroon trace).

As indicated in the 2D COSY spectrum shown in **Figure 3**, this proton is J-coupled to three different protons on nearby carbon atoms. Depending on the amount of time the J-coupling is allowed to evolve during the COSY experiment, some peaks may appear more intense than others. For example, when the timing is optimized for a J-coupling of 11 Hz (**Figure 8, green trace**), the proton on carbon 11 is most intense at 4.1 ppm in the resulting 1D Selective COSY. As shown in **Figure 1**, these protons reside on neighboring carbon atoms, thus resulting in a three-bond J-coupling. Protons that are farther than three bonds away generally exhibit smaller coupling. Thus with the delay optimized for a J-coupling of 3.5 Hz (**Figure 8, teal trace**), the protons on carbons 16 near 3.6 ppm and 22 at 3.1 ppm are more clearly observable in the 1D Selective COSY. In both cases, the correlated peaks in the 1D Selective COSY have antiphase lineshapes. This can complicate analysis if many correlated peaks are present in varying intensities. One possible solution is to run a 1D selective TOCSY instead, where the correlated peaks have in phase lineshapes, as shown in the **purple trace of Figure 8**. In this case, the mixing time will determine the extent to which the J-coupled partners are observed. With a 120 ms mixing time, all the correlated peaks observed in the separate COSY experiments are also observable, along with some additional long range, low intensity correlations to protons on carbons 23 (2.27 and 1.54 ppm), 19 (1.35 ppm), 12 and 13 (3.87 ppm), as well as cleaner observation of the 16" proton resonances near 2.8 and 2.6 ppm.

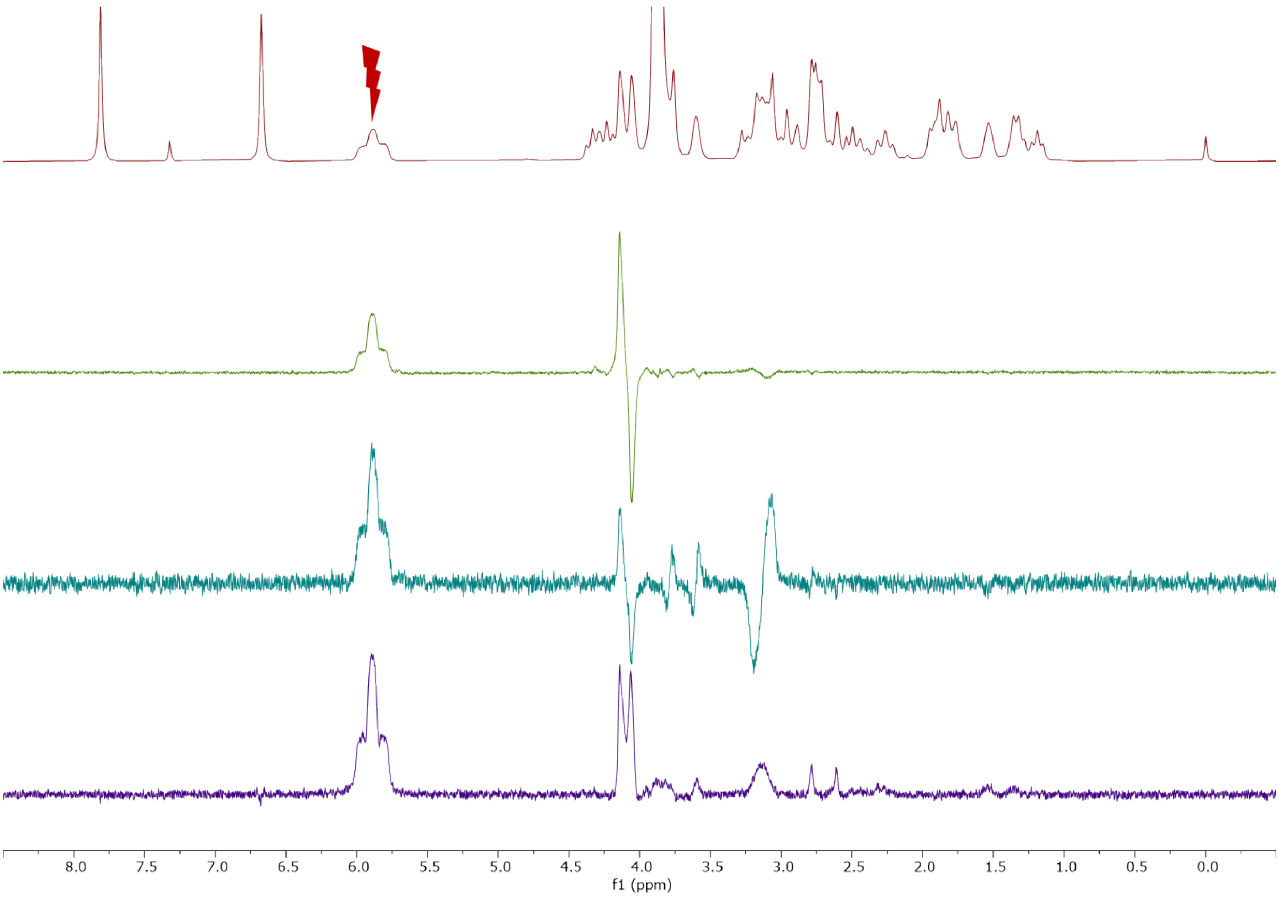

Figure 8 The 1D ^{1}H spectrum (**maroon trace**) showing the peak used for selective excitation (5.7 ppm) in the subsequent experiments. Two 1D Selective COSY experiments with timing optimized for two different values for the J-coupling constant, 11 Hz (**green trace**) and 3.5 Hz (**teal trace**), were acquired with 8 scans and 2.5 minutes experiment time. Lastly a 1D Selective TOCSY with 120 ms mixing time (purple trace) was acquired with 32 scans and 7.5 minutes experiment time.

This set of experiments demonstrates the vast potential of the Fourier 80 to perform NMR analysis from the benchtop: from routine 1D ^{1}H or ^{13}C spectra for structural analysis, to selective heteronuclear and homonuclear experiments for addressing more specific or complex structural questions. In addition to the flexibility offered by a system with both ^{1}H and ^{13}C channels, the use of gradients, shaped pulses, and NUS enable fast, efficient, and reliable data acquisition.

References:

1. Lu, L.; Huang, R.; Wu, Y.; Jin, J.-M.; Chen, H.-Z.; Zhang, L.-J.; Luan, X. Brucine: A Review of Phytochemistry, Pharmacology, and Toxicology. Frontiers in Pharmacology 2020, 11.

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