Structural elucidation of indole alkaloids - Strychnine and Brucine

Both Strychnine and Brucine (2,3-Dimethoxystrychnidin-10-one) belong to the versatile structural family of alkaloids. In detail they both belog to the class of terpene indoles as they both incorporate the structural motif of an indole. Here the nitrogen differentiates these class of compounds from e.g. steroide alkaloids. Both compounds are crystalline solids with high toxicity, where Strychine is the much more potent one in comparison. Strychnine and Brucine are both present in nature, but have as well been successfully synthesized by total synthetic approaches. Figure 1 shows the ^{1}H NMR spectrum of a 100 mM Strychnine sample in $CDCl_3$ measured in a single scan taking 15 seconds to acquire.

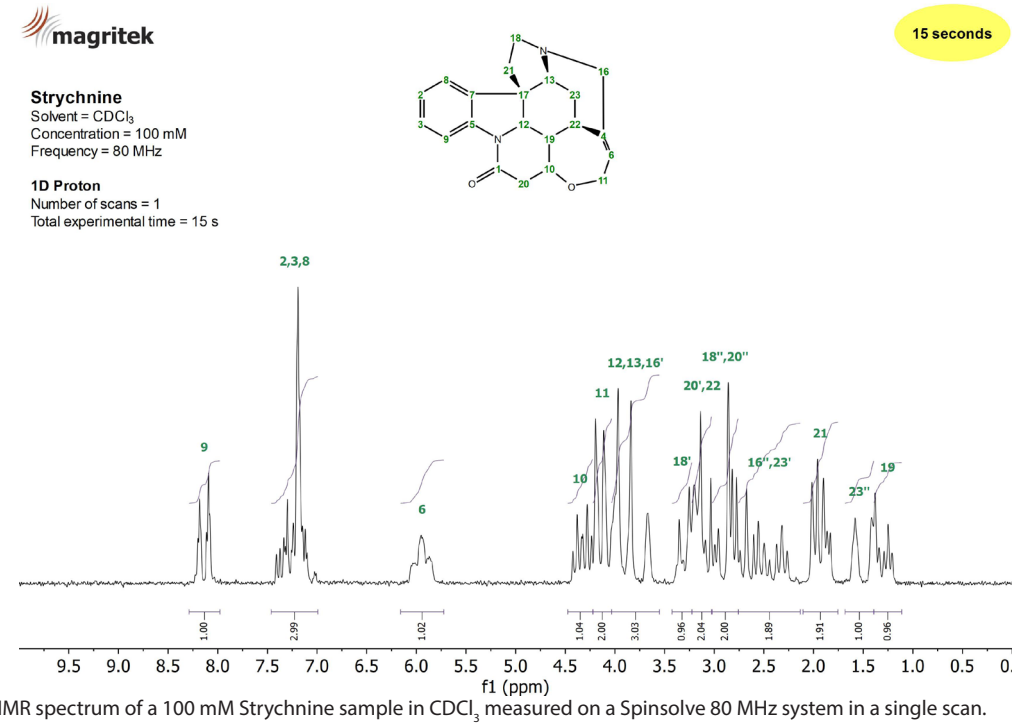

Figure 1: ^{1}H NMR spectrum of a 100 mM Strychnine sample in $CDCl_3$ measured on a Spinsolve 80 MHz system in a single scan.

2D COSY

The 2D COSY experiment allows one to identify coupled ^{1}H nuclei as they generate cross peaks out of the diagonal of the 2D data set. In Figure 2 a large number of cross peaks can be nicely observed. For example, the aromatic protons at positions 2,3,8 and 9 (light green) couple with each other. In addition, the couplings between proton 6 and protons 11 (pink), protons 16 (orange) and proton 22 (blue) can be nicely observed.

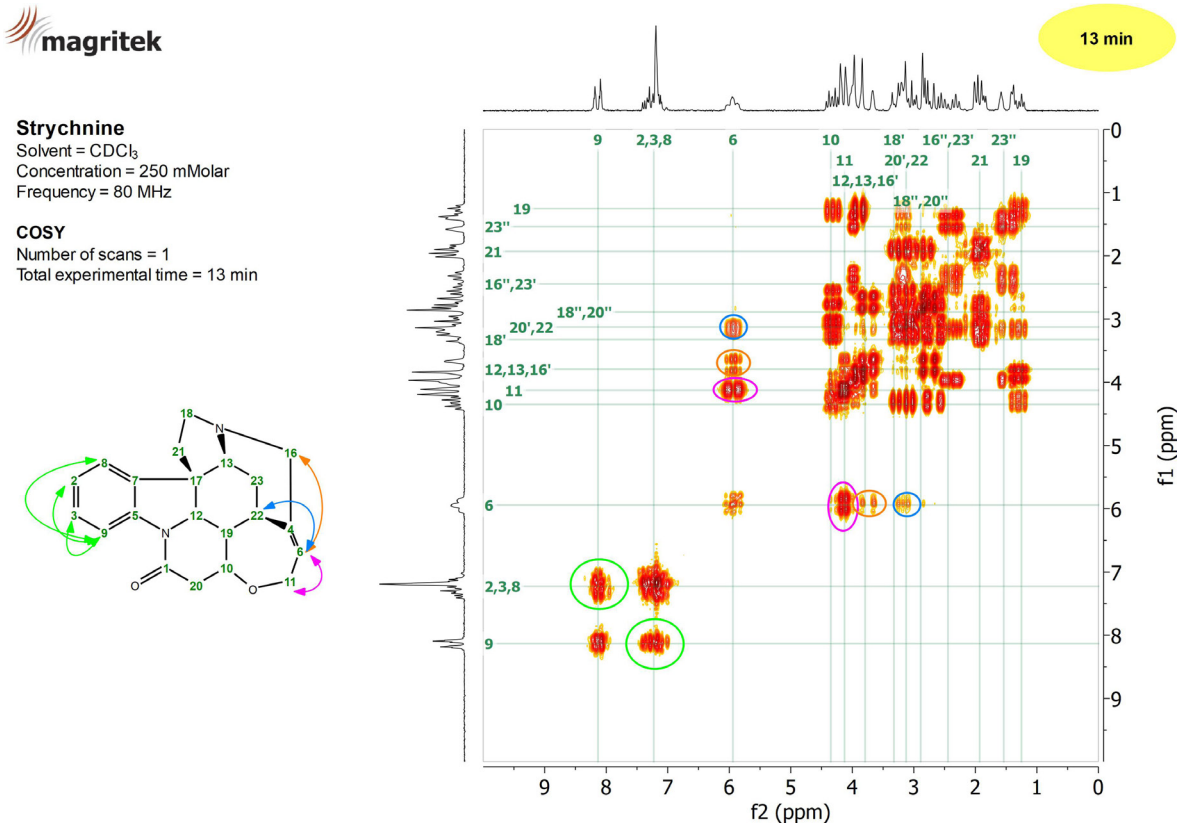

Figure 2: ^{1}H 2D COSY experiment of a 250 mM Strychnine sample in $CDCl_3$ acquired in 13 minutes on a Spinsolve 80 MHz system.

^{13}C Spectrum

Figure 3 shows the ^{13}C NMR spectrum of 430 mM Strychnine in $CDCl_3$ acquired using NOE polarization transfer from ^{1}H to ^{13}C and ^{1}H decoupling. The 1D Carbon experiment using NOE is sensitive to all ^{13}C nuclei in the sample. It clearly resolves all the expected resonances.

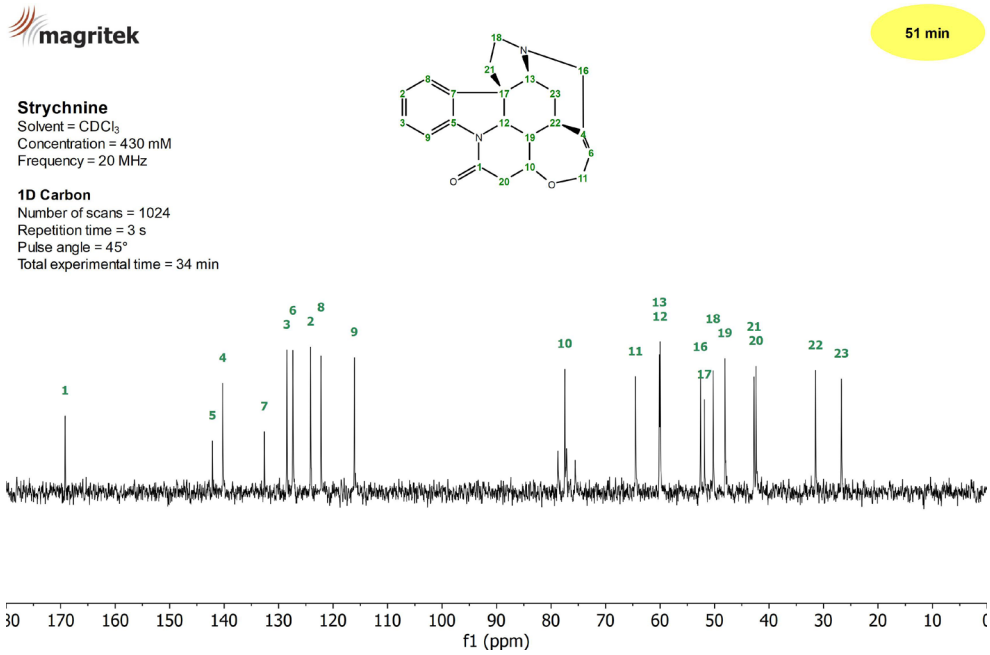

Figure 3: ^{13}C NMR spectrum of a 430 mM Strychnine sample in $CDCl_3$ measured on a Spinsolve 80 MHz system in 51 minutes.

2D HSQC-ME and 2D HMBC

The HSQC and HMBC are powerful sequences widely used to correlate the one-bond coupled ^{1}H-^{13}C nuclei and long range ^{1}H-^{13}C, respectively. The Spinsolve is equipped with a multiplicity edited version (HSQC-ME) of this method. It provides the editing power of the DEPT-135 sequence, which is useful to differentiate between the signals of the CH_2 groups (blue) from the CH and CH_3 groups (red). Thanks to the higher sensitivity of these sequences, compared to direct detected ^{13}C experiments, samples with lower concentrations can be measured in relatively short times. Figure 4 shows the HSQC-ME (left) and HMBC (right) spectra of a 430 mM Strychnine sample in $CDCl_3$ acquired in 18 minutes and 35 minutes, respectively. The HSQC spectrum resolves all coupled ^{13}C and allows one to resolve the ^{1}H signals that overlap in the 1D proton spectrum. On the other hand, the HMBC shows the long-range correlations that are useful to identify and assign the different peaks in the spectrum. As an example, we marked on the HMBC spectrum the cross peaks of protons 11 with carbons 4, 6 and 10 (the sequence shows the correlation with quaternary carbons, too).

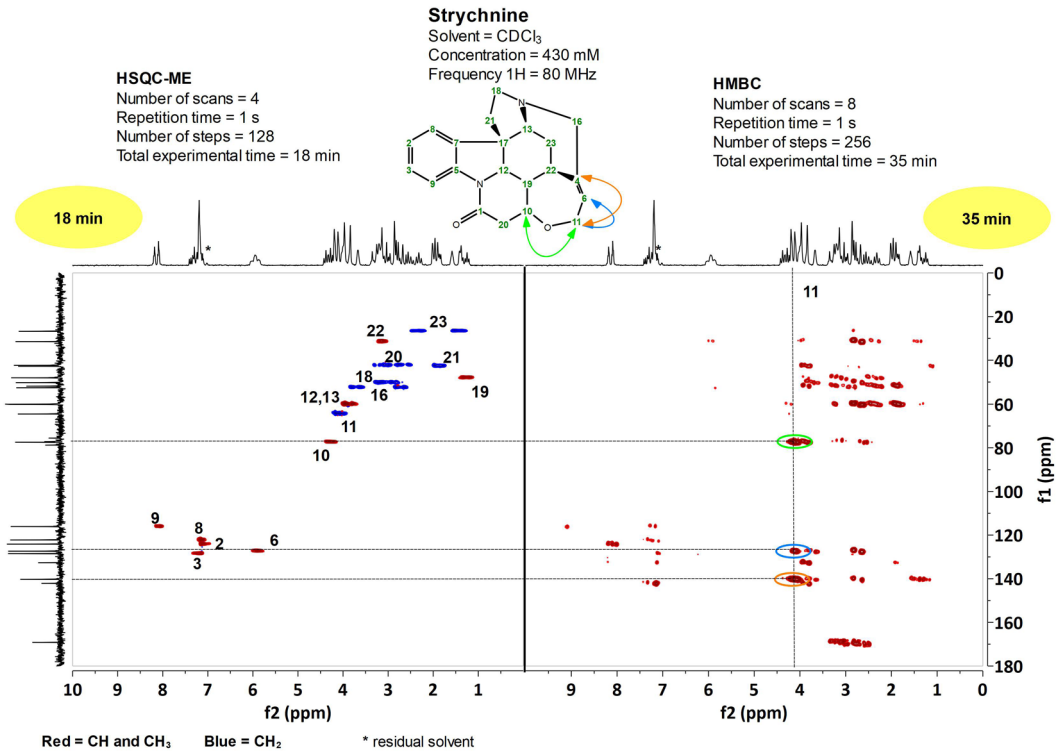

Figure 4: 2D HSQC-ME (left) and HMBC (right) spectra of a 430 mM Strychnine sample in $CDCl_3$ measured on a Spinsolve 80 MHz system.

Figure 5 shows the ^{1}H NMR spectrum of a 100 mM Brucine sample in $CDCl_3$ measured in a single scan taking 15 seconds to acquire.

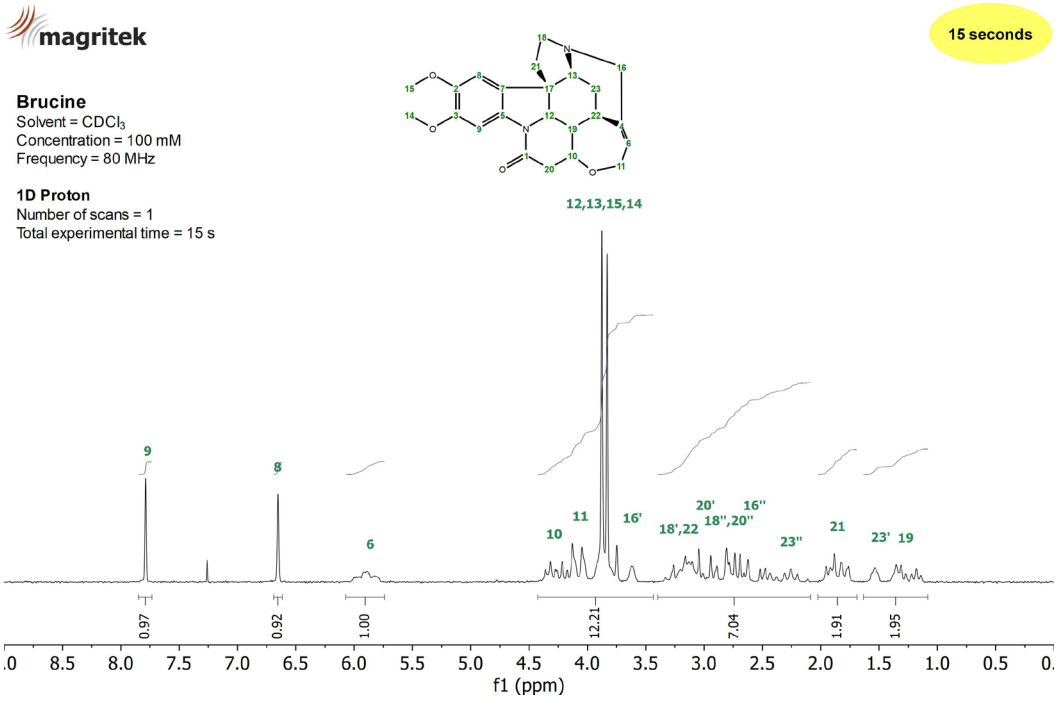

Figure 5: ^{1}H NMR spectrum of a 100 mM Brucine sample in $CDCl_3$ measured on a Spinsolve 80 MHz system in a single scan.

2D COSY

The 2D COSY experiment allows one to identify coupled ^{1}H nuclei as they generate cross peaks out of the diagonal of the 2D data set. In Figure 6 a large number of cross peaks can be nicely observed. For example, the protons at position 6 and 11 (light green) couple with each other. Furthermore, proton 19 couples with proton 10 (light blue), 12 (orange) and 20 (pink). In addition, the couplings between protons 8 and 9 (dark blue) as well as the couplings of protons 8 and 9 with protons 14 and 15 (dark green) can be nicely observed.

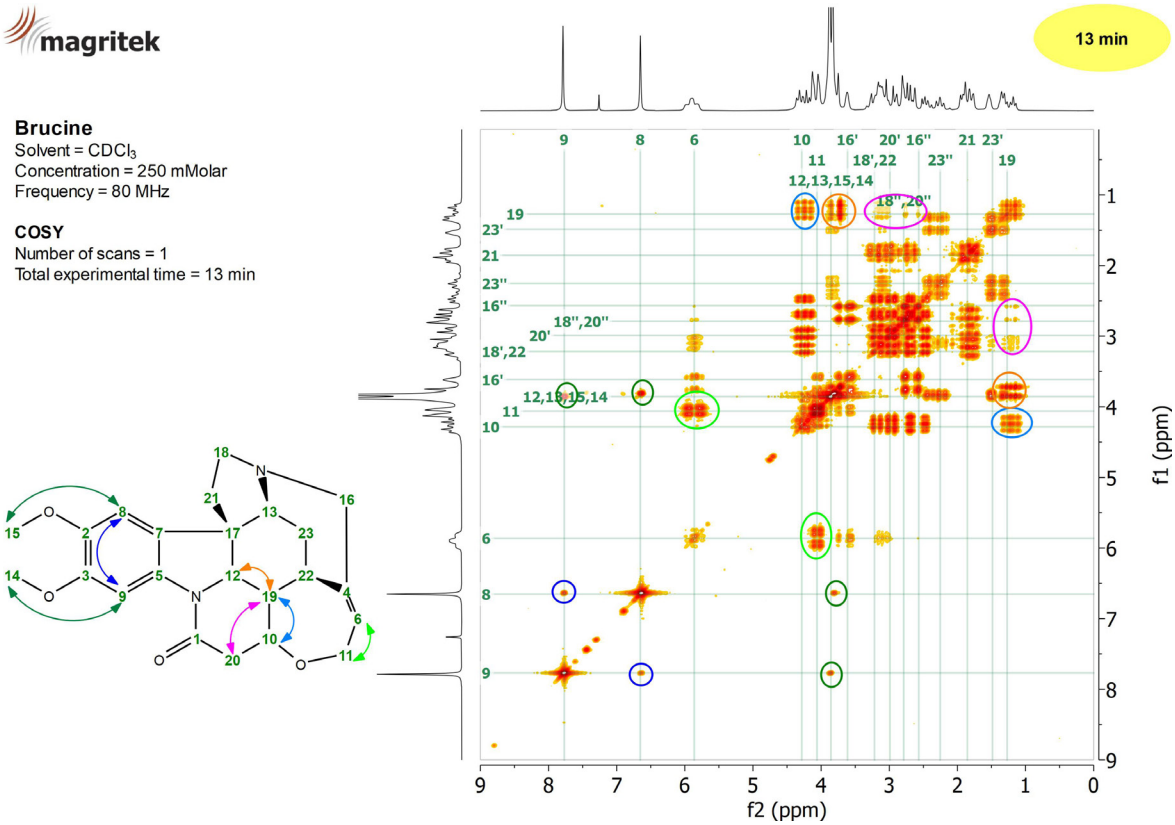

Figure 6: ^{1}H 2D COSY experiment of a 250 mMolar Brucine sample in $CDCl_3$ acquired in 13 minutes on a Spinsolve 80 MHz system.

^{13}C Spectrum

Figure 7 shows the ^{13}C NMR spectrum of 1 M Brucine in $CDCl_3$ acquired using NOE polarization transfer from ^{1}H to ^{13}C and ^{1}H decoupling. The 1D Carbon experiment using NOE is sensitive to all ^{13}C nuclei in the sample. It clearly resolves all the expected resonances.

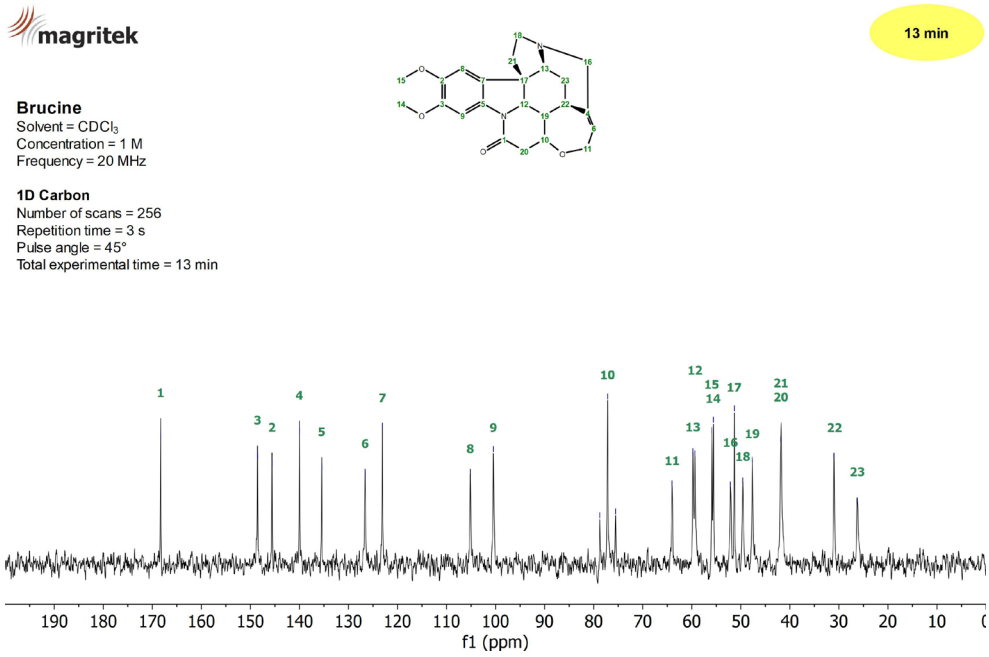

Figure 7: ^{13}C NMR spectrum of a 1 M Brucine sample in $CDCl_3$ measured on a Spinsolve 80 MHz system in 13 minutes. (top).

2D HSQC-ME and 2D HMBC

The HSQC and HMBC are powerful sequences widely used to correlate the one-bond coupled ^{1}H-^{13}C nuclei and long range ^{1}H-^{13}C, respectively. The Spinsolve is equipped with a multiplicity edited version (HSQC-ME) of this method. It provides the editing power of the DEPT-135 sequence, which is useful to differentiate between the signals of the CH_2 groups (blue) from the CH and CH_3 groups (red). Thanks to the higher sensitivity of these sequences, compared to direct detected ^{13}C experiments, samples with lower concentrations can be measured in relatively short times. Figure 8 shows the HSQC-ME (left) and HMBC (right) spectra of a 300 mM Brucine sample in $CDCl_3$ acquired in 18 minutes and 35 minutes, respectively. The HSQC spectrum resolves all coupled ^{13}C and allows one to resolve the ^{1}H signals that overlap in the 1D proton spectrum. On the other hand, the HMBC shows the long-range correlations that are useful to identify and assign the different peaks in the spectrum. As an example, we marked on the HMBC spectrum the cross peaks of proton 8 with carbons 2, 3, 5, 7, 9 and 17 (the sequence shows the correlation with quaternary carbons, too).

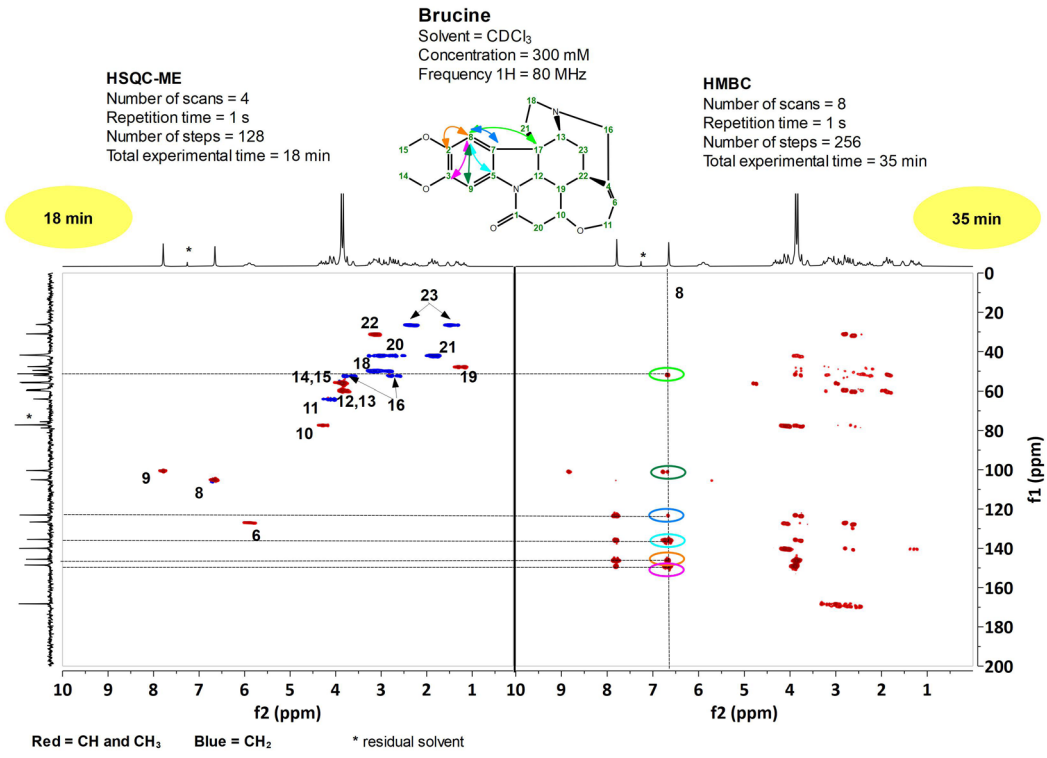

Figure 8: HSQC-ME (left) and HMBC (right) spectra of a 300 mMolar Brucine sample in $CDCl_3$ showing the correlation between the ^{1}H (horizontal) and ^{13}C (vertical) signals.