Need for high resolution 2D spectra

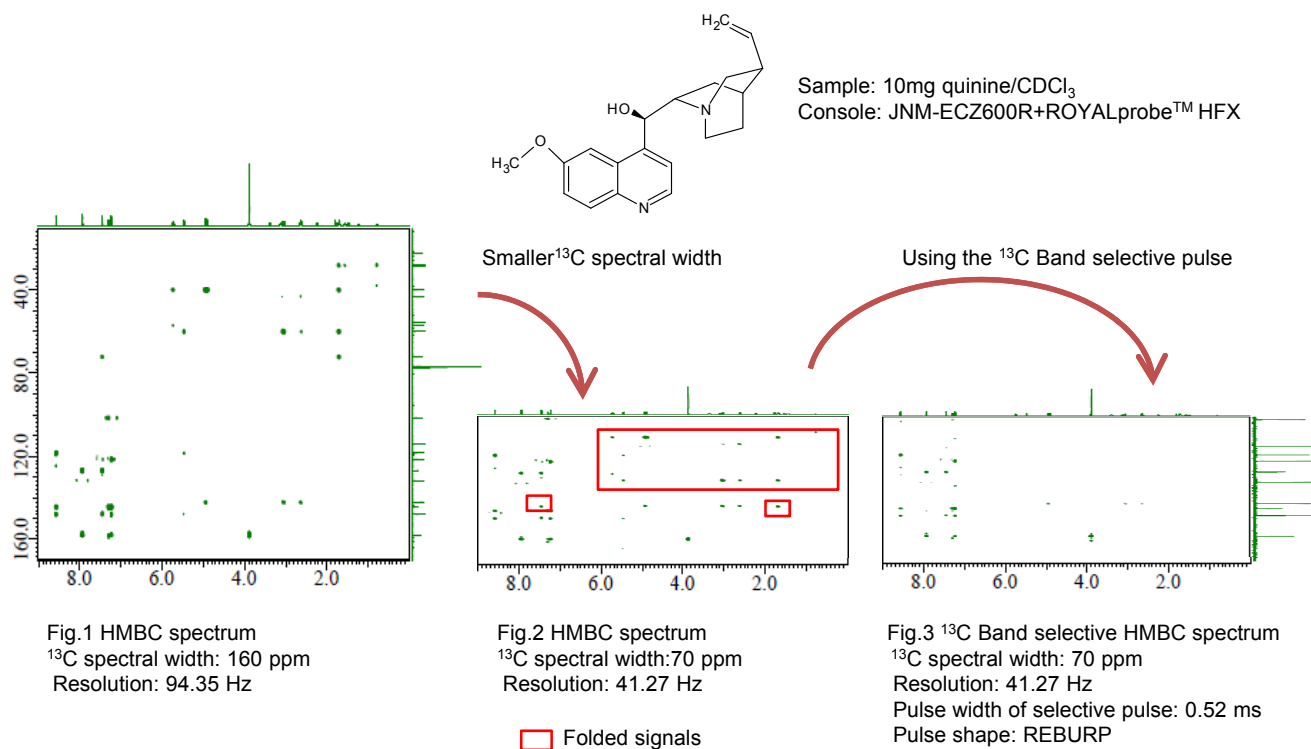## Product used : Nuclear Magnetic Resonance (NMR)

^{13}C NMR spectra provide wide range chemical shift, and it suggests that can easily distinguish each signals. But carbon resolution of 2D spectra such as HSQC and HMBC is worse than 1D ^{13}C spectra due to small data points. In order to analyze a compound with close ^{13}C chemical shifts, a high resolution 2D spectrum is required frequently. In this document, some improvements to distinguish each signals on ^{13}C axis of 2D hetero nuclear experiments are presented.

Reducing spectral width

In order to improve the resolution on the indirect dimension, ^{13}C spectral width for 2D experiments should be set to the actual signal region. (Fig.1)

When limited region spectra would be enough to analysis, the higher resolution spectra will be available with the smaller spectra width parameter. However many signals come from the outside of the required region for the indirect dimension (fold), and it makes complicated and/or overlapping spectra frequently (Fig.2 red boxes).

In that case, by using selective pulse for 2D NMR experiments, simple high resolution spectra without folded signals will be collected (Fig.3).

Sample: 10mg quinine/CDCl$_3$
Console: JNM-ECZ600R+ROYALprobe^{TM} HFX

Smaller ^{13}C spectral width

Using the ^{13}C Band selective pulse

Fig.1 HMBC spectrum
^{13}C spectral width: 160 ppm
Resolution: 94.35 Hz

Fig.2 HMBC spectrum
^{13}C spectral width: 70 ppm
Resolution: 41.27 Hz

☐ Folded signals

Fig.3 ^{13}C Band selective HMBC spectrum
^{13}C spectral width: 70 ppm
Resolution: 41.27 Hz
Pulse width of selective pulse: 0.52 ms
Pulse shape: REBURP

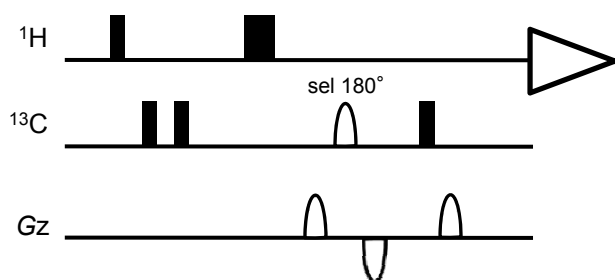## Pulse sequence of ^{13}C band-selective HMBC

In this sequence, hard180 pulse is replaced by selective pulse.
We can get only limited range's correlation by using ^{13}C selective pulse. (Fig.4)

Fig.4 Pulse sequence of ^{13}C band-selective HMBC

Increasing data points

If data points increase 2 or 4 times for indirect axis (^{13}C axis), digital resolution also improves 2 and 4 times. But experiment time also increases 2 and 4 times . Moreover , if carbon FID is short-lived then signal to noise ratio would be poor.

The Non-Uniform-Sampling (NUS) is an effective technique to shorten experiment time for samples in adequate concentration. Fig.7 shows NUS version of the same experiment as in Fig.1 and Fig.3 but with 4 times more total points in carbon dimension and 25% NUS sampling. As a result, NUS spectrum shows 4 times higher resolution than spectrum in Fig.3 for the same experiment time.

Quinine has two ^{13}C signals with chemical shifts at 147.63ppm and 147.83ppm. It is difficult to distinguish them in HMBC spectrum of Fig.5. By contrast, it becomes easy to distinguish them due to improved resolution in spectra of Fig.6 and Fig.7.
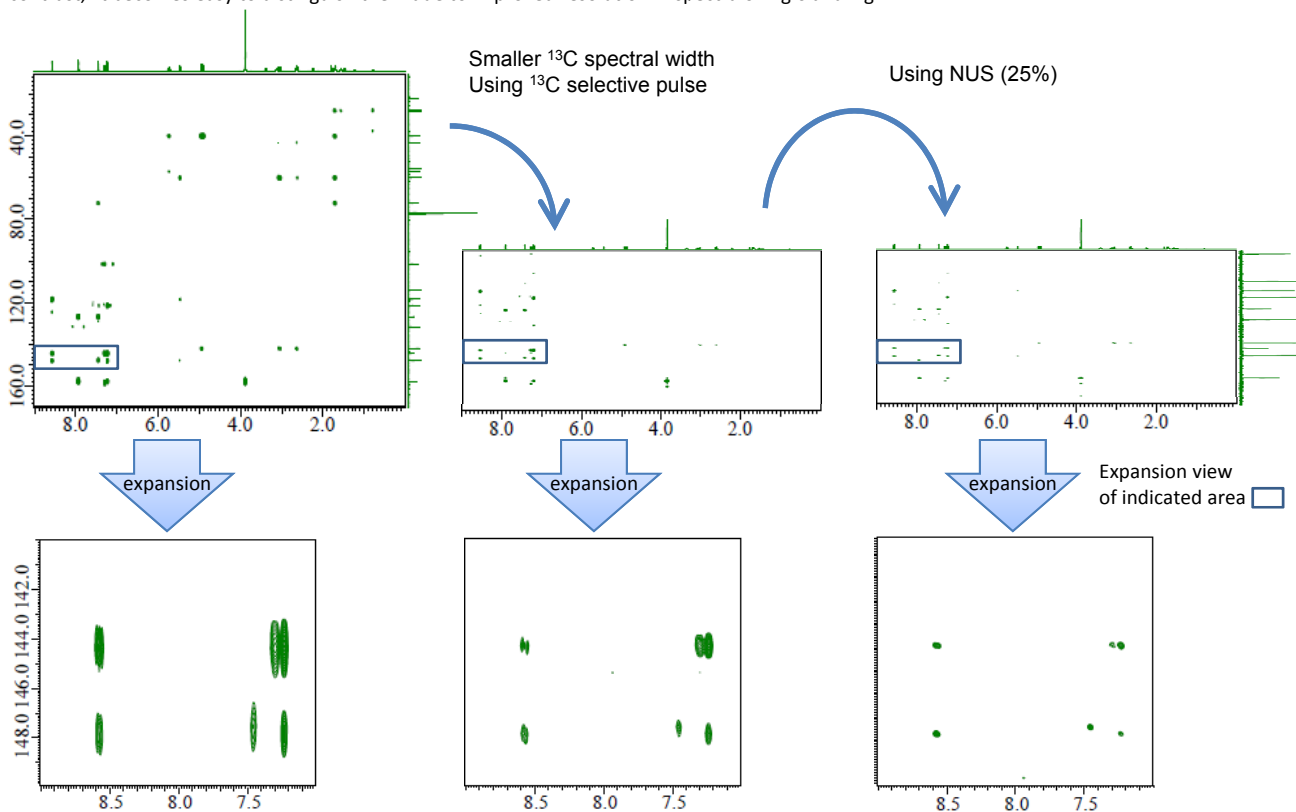
Smaller ^{13}C spectral width
Using ^{13}C selective pulse

Using NUS (25%)

expansion expansion expansion

Expansion view
of indicated area ▭

Fig.5 HMBC spectrum
^{13}C spectral width: 160ppm
Data points: 256
Experiment time: 30 min
Resolution: 94.35 Hz

Fig.6 ^{13}C Band-selective HMBC spectrum
^{13}C spectral width: 70ppm
Data points: 256
Experiment time: 30 min
Resolution: 41.27 Hz

Fig.7 ^{13}C Band-selective HMBC spectrum (NUS)
^{13}C spectral width：70ppm
Data points: 1024
NUS: 25%
Experiment time : 30 min
Resolution : 10.31 Hz

Non-Uniform-Sampling (NUS)

NUS is the sampling method which reduces the indirect dimension data points by collecting data points inhomogeneously. This data collection method would reduce experiment time, and it would give higher resolution spectrum for the same experiment time as indicated in this document. Not collected data points are reconstructed after data acquisition. Reconstructed FID data is available for the conventional data processing.

Fig.8 (Uniform sampling) and Fig.9 (NUS + reconstruction) are diagrams of FID in indirect dimension. The collected data points are the same each other, and measurement times are also the same. But NUS data points increase by 4 times due to reconstruction. Because resolution is proportional to data points, NUS data show 4 times higher resolution than conventional data.
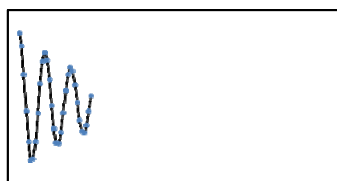
· Sampling points
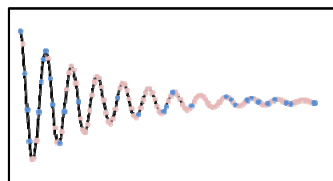· Reconstructed points

Fig.8 Uniform sampling

Fig.9 NUS + reconstruction (25% data collection)