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carbon1d.nan

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NAN-KB UGA[1]

[1]Network for Advanced NMR (NAN)

Edison Lab

NAN-KB UGA
Network for Advanced NMR

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Protocol status: Working
We use this protocol and it's working

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Disclaimer

This protocol is part of the knowledge base content of **NAN:** The Network for Advanced NMR (**https://usnan.nmrhub.org/**)
Specific filenames, paths, and parameter values apply to spectrometers in the NMR facility of the Complex Carbohydrate Research Center (CCRC) at the University of Georgia.

Abstract

This protocol describes running a 1D carbon experiment with proton decoupling using the Bruker pulseprogram 'zgpg30'. This sequence sets the carbon pulse width to get a tip angle of 30 degrees for more rapid acqusition with a shorter relaxation delay.
The default settings put the transmitter at 100 ppm with a spectral window of 225ppm. It will show all protonated and unprotonated carbons.
Signal intensity will be a function of degree of protonation due to NOE, and relaxation times of individual carbon nuclei.
This pulse program can be used with either natural abundance 13C samples or 13C-isotopically enriched samples.

Guidelines

This protocol assumes a sample is properly loaded into the magnet. Potential **users must be trained** in the proper handling of samples for spectrometers at the CCRC NMR facility.

See protocols "Loading_a_sample_manually.nan" or "Loading_a_sample_with_SampleJet.nan" for reference.

Safety warnings

- This pulse sequence uses continuous pulsing of the 1H channel at low power for proton decoupling: double check that the default value for the CPD decoupling is at least 16dB lower than the high-power pulse (P1) pulse power.

Before start

A sample must be inserted in the magnet either locally by the user after training (see Guidelines) or by facility staff if running remotely.

You must first collect a proton 1D spectrum on a sample that is shimmed and has a calibrated 90 degree proton pulse, according to the protocol **noesypr1d_metab.nan.**

Create carbon1d experiment file

1

Starting from the 1D proton data set, click on Acquire -> 'Create Dataset' button to open dataset entry box.
It should increment the EXPNO to the next integer (e.g. 2)

1.1

Edit the Title text box with pertinent information and indicating 1D carbon observation.

1.2 **Check 'Read parameterset' box, and click Select.**

For standard NAN parameter sets, change the Source directory at upper right corner of the window:
Source = /opt/NAN_METAB/par

Click 'Select' to bring up list of parameter sets. Select CARBON1D_xxx.par where xxx could be 'br900_urine', etc.

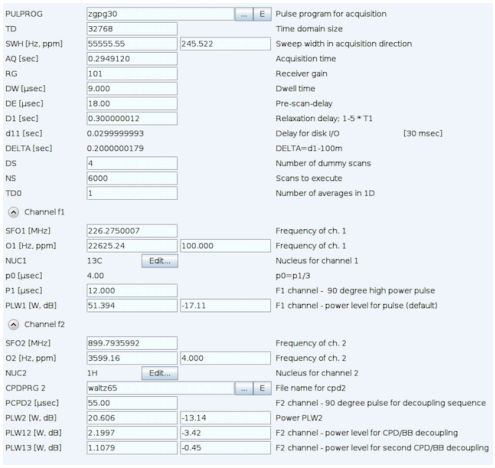
If using the standard Bruker parameter library, the equivalent parameter set is 'C13CPD'.

1.3

Click OK at bottom of window to create the experiment directory.
It will be the active experiment in the acquisition window and should now be listed in your data directory.
The actual pulse sequence is named 'zgpg30'.

2 **Load and adjust typical parameters.**

Select the 'Acqpars' tab (or type ased) which brings up the page of common and pulse program specific parameters.
The complete list of parameters can be accessed by clicking on the 'A' at upper left menu, or typing eda.
There should be parameters for both 13C (f1) and 1H (f2) channels.
See step 3.2 for typical parameters to change.



screenshot from 800MHz (CCRC BR800) - values will depend on spectrometer

2.1 **Load probe and solvent dependent parameters**.

Clicking the menu item 'Prosol' , or typing 'getprosol' in the command line will load the 13C parameters, HOWEVER it will also load the DEFAULT proton parameters, NOT the calibrated pulse width obtained from the proton data set in step 1.
Instead type the following command:

Command

getprosol 1H [calibrated P1 value] [standard 1H power level]

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getprosol 1H [calibrated P1 value] [standard 1H power level]
```

where [calibrated P1 value] is the 90 degree proton pulse width obtained with 'pulsecal' (step 1)
and the [standard 1H power level] is the power level set for this proton pulse (step 1, e.g. found in parameters of proton dataset EXPNO 1). This power level is established when the probe is installed and will be different for different spectrometers and probes.
e.g. ' getprosol 1H 7.5 -13.14' sets the P1 value to 7.5 usec at -13.14 dB attenuation (= equivalent to power rating).

Loading parameters in this way ensures that all proton pulse widths (e.g. CPD decoupling) are correctly calculated based on the correct proton 90 degree pulse.

2.2 Change other parameters as needed.

TD or AQ: increase for better digital resolution (1-3 seconds is adequate).
SWH: carbon spectral window, typically at least 200 ppm
NOTE: changing SWH will change AQ.

D1: 1-3 seconds is normal; increase to 5 seconds for very slowly relaxing quaternary carbons
DS: dummy scans that are not recorded for steady state equilibration; at least 4
NS: increase for increased signal to noise (S/N increases as √NS);
-recall for natural abundance 13C the effective concentration is only 1.1% in addition to inherent lower sensitivity of 13C vs 1H.
-if you do not know how many scans to acquire for natural abundance material, set the number to be large (e.g 1800 for ~2hr), and look signal accumulation (see step 4)

O1: carbon frequency offset; center of spectral window. Typically 100 ppm.
O2: proton frequency offset, center of proton region (e.g. 5 ppm for all protons, 3ppm for aliphatic only)
TD0: This is a safe method to save data during long acquisitions, rather than wait till the end or manually use 'tr' and run the risk of it being deleted.
Set **TD0=some_number**, and reset NS since now **Total_NS = TD0*NS** and most importantly your data will be saved every NS scans.
e.g. total_NS needed = 1024, set NS=16 and TD0 =64.

2.3 **Adjust receiver gain.**
Click on 'Gain' in the main topspin Acquire menu or type 'rga'.
Typically at maximum value (101 or 203) for weak 13C signal.

3 **Acquire and process data.**

Click on 'Run' in Topspin Acquire menu, or type 'zg'.

If NS is large and you haven't used the TD0 option (see above), you can look at the data at any time while it is still acquiring by typing 'tr': just type 'tr' to save after the next scan, or e.g. 'tr 64' for a look at 64 scans, , wait a few seconds, then execute step 4.1. to see spectrum.

3.1 Click on 'Proc.Spectrum' on the Topspin Process menu (requires data written to disk - see above).
This will execute an automated processing macro.
If you are satisfied with the signal to noise of the data, you can halt the acquisition and save the data by typing 'halt' or clicking on the small square icon in the menu. **DO NOT click on 'STOP'** since that will just kill the acquisition without writing the data to disk. See above 3.2 and 4 for ways to write data to disk during acquisition to avoid potential loss of data.