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HSQC_CT_13C_ali.nan

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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 2D constant-time ^{13}C HSQC pulse sequence with sensitivity enhancement and echo-antiecho gradient coherence selection, optimized for high resolution in the aliphatic region. This produces a 2D phase-sensitive ^{13}C-^{1}H correlation spectrum that displays signals for each aliphatic carbon-proton pair.

Required isotope labeling: U-^{15}N,^{13}C or U-^{13}C. Not suitable for samples with uniform ^{2}H labeling due to starting with aliphatic ^{1}H polarization. Application to samples at natural abundance is possible but would require high concentration, sensitive probes and lengthy signal averaging.

Optimal MW is less than 25 kDa.

Field strength preference: High B_0 fields are preferred for better signal dispersion.

This pulse sequence can be used for:

- resonance assignment of backbone and side-chain aliphatic ^{1}H and ^{13}C resonances
- spin system identification
- routine sample screening and stability monitoring
- chemical shift perturbation studies due to ligand binding, paramagnetic relaxation or pseudo-contact shifts
- optimization of aliphatic ^{13}C offset and spectral width for use with 3D ^{13}C-edited NOESY/TOCSY, HCCH, and other experiments.
- anchoring of 3D spectra (^{13}C-edited NOESY/TOCSY, HCCH) during interactive visual analysis

It uses the pulseprogram 'hsqcctetgpsisp_ali.nan' (hsqc= heteronuclear single quantum correlation; ct=constant time; et=echo-antiecho; si=sensitivity enhanced; sp=shaped 180 pulses) modified from the original Bruker library sequence *hsqcctetgpsisp* with the following changes:

- Simultaneous 180° ^{13}C and ^{15}N pulses replaced with "staggered" sequential pulses to reduce lock perturbations and avoid t1 noise artifacts on high-Q probes.
- Refocusing Q3 shaped pulse on ^{13}C in the middle of CT evolution period replaced with a rectangular 180° pulse ("soft" rectangular pulse with a null excitation at ^{13}CO, or a hard pulse, depending on field strength). This allows uniform phasing to a pure absorptive line shape across the entire ^{13}C range.
- ^{15}N 180° decoupling pulse during ^{13}C CT evolution replaced with a broadband 90-180-90 composite pulse
- Introduced additional gradient pulses p16 and p19

Note: specific parameter values illustrated below may differ depending on the facility and spectrometer.

Guidelines

The number of directly acquired points (**2 TD**) should be set so the acquisition time $t_{2,max}$ (**2 AQ**) is between ~50 ms (for larger proteins ~25 kDa) and ~120 ms (for smaller proteins). Longer times may cause excessive probe and sample heating during ^{13}C decoupling, and resolve undesirable $^{2,3}J^{H,H}$ splittings.

Total constant-time delay is equal to 2∗**D23**, and is normally set to multiples of $(1/^{1}J_{CC})$. Here $^{1}J_{CC}$ is assumed to be 37.5 Hz, though the true splittings may vary slightly from site to site. The most common settings are $(1/^{1}J_{CC})$ and $(2/^{1}J_{CC})$, corresponding to **D23** of 0.0133 and 0.0266 s, respectively. In the former case peak signs will depend coupled spin multiplicity (even or odd number of covalently linked ^{13}C spins), while in the latter case all peaks have the same sign.

Since the F1(13C) is a constant-time dimension, number of acquired points **1 TD** should be should be set to the largest possible value for optimal S/N and resolution. The maximum **1 TD** value is 2*int(d20/in20), where d20 is the decremented delay, and in20 is the decrement value. Both d20 and in20 are computed internally according to pulse program code and their values are shown in ased view. Note that in0 = in20 = 1(2∗SW(13C)). In TopSpin 4.x the ased command would produce a warning if **1 TD** is set too large and reset the internal *td2* parameter to the maximum possible value.

NUS sampling is usually not required for 2D experiments, since time savings are small, unless running multiple 2D experiments. If using NUS keep sampling amount ~50%.

Before start

A sample must be inserted in the magnet either locally by the user after training or by facility staff if running remotely.
This protocol requires a sample is locked, tuned/matched on ^{1}H, ^{13}C and ^{15}N channels, and shimmed. At a minimum, ^{1}H 90° pulse width and offset O1 should be calibrated and a 1D proton spectrum with water suppression has been collected according to the protocol **PRESAT_bio.nan.**

It is recommended to calibrate ^{1}H carrier offset, ^{1}H H_2O selective flip-back pulse, as well as ^{1}H, ^{13}C, and ^{15}N 90° pulse widths using the "Optimization" tab of **bioTop**. Alternatively, ^{1}H 90° pulse width and offset can be calibrated using other methods, such as **pulsecal** or **calibo1p1**. Additional parameters, like ^{15}N and ^{13}C offsets and spectral width can be either optimized or manually entered in the "Optimization" tab of **BioTop**.
Note that since BioTop optimizations are saved in the dataset folder, all experiments should be created under the same dataset name when using BioTop for acquisition setup.

Familiarize yourself with the general workflow for NMR study of a protein sample is outlined in protocol "Aquisition Setup Workflow, Solution NMR Structural Biology".

Create aliphatic 13C CT-HSQC experiment

1 Start with existing Dataset containing 1D proton data in EXPNO 1.
(see step 1)

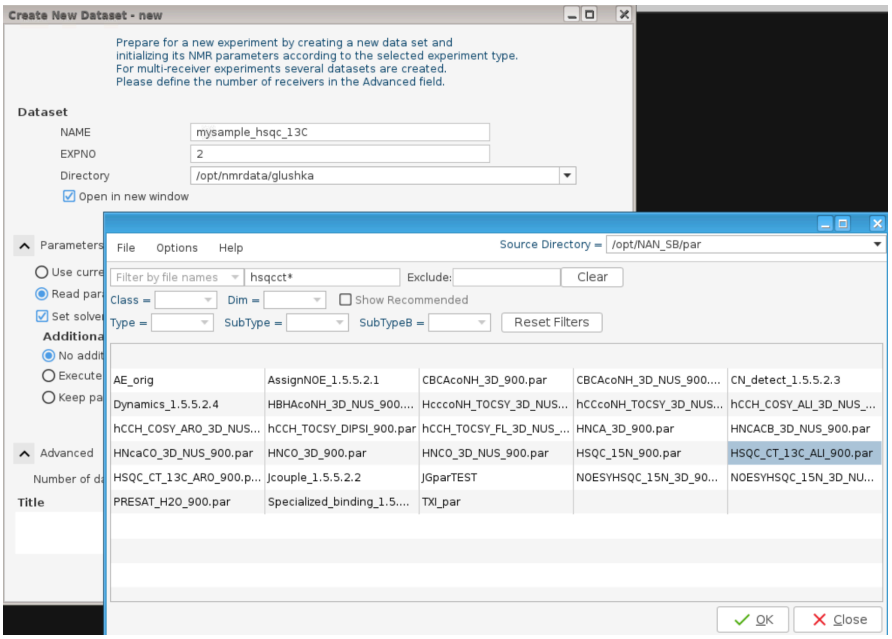
1.1 Click on Acquire → 'Create Dataset' button to open dataset entry box.
or type **edc** command.

Dataset: The Name defaults to the one used for the previous 1D dataset.
If a different name is desired, fill this out.
The EXPNO should increment by +1
Directory should be the same as preliminary 1D.

The Title text box will be the same as for EXPNO 1.
Edit to designate an HSQC pulse program and add other details as appropriate.

1.2 Select starting parameter set:
Check 'Read parameterset' box, and click Select.

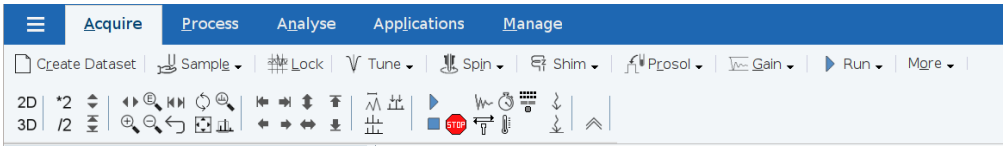
For standard NAN parameter sets, change the Source directory at upper right corner of the window:
Source = /opt/NAN_SB/par
Select HSQC_CT_13C_ALI_xxx.par, where xxx=900,800 or 600*.



* parameter set name may differ depending on spectrometer

1.3 Click OK at bottom of window to create the new EXPNO directory.
It will be the active experiment in the acquisition window and should now be listed on your data browser.

1.4 **Tune Carbon (and Nitrogen) channels if not done.**
Return to the Acquire menu and click Tune (or type atma on command line).



This will start tuning of the nitrogen channel, then the carbon channel, followed by a retuning of the proton channel (which should not change).

Load pulse calibrations: use getprosol (step 2.1) or bioTop (step 2.2)

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Note

Loading the HSQC_CT_13C_ALI_xxx.par parameter set enters the default parameters into the experiment directory. While a good starting point, they may not be fully optimal or accurate for your particular sample or spectrometer hardware. The probe- and solvent-specific parameters, specifically the ^{1}H 90° pulse length, and possibly the ^{13}C and ^{15}N 90° pulse lengths, along with other dependent pulse widths and powers may need to be updated.

There are two ways of automatically updating an entire range of experimental parameters. The first is using **getprosol** command (step 2.1), which only updates pulse widths and power levels without altering other parameters, such as spectral widths and offsets. This method is suitable for reproducing existing experiments or parameters sets with minimal variations.

The second method utilizes the **BioTop** module of TopSpin (step 2.2), and can load additional experimental parameters, such as spectral widths, offsets, and number of time-domain points. These additional parameters are set according to calibrations or definitions within the 'Optimization' tab of the BioTop GUI and the corresponding XML description files (*bt_hsqcctetgpsisp_ali.nan.xml* in this case). This method has a lower dependency on the particular settings of the starting parameter set, and is suitable for setting up experiments from scratch. With this method nearly all important acquisition parameters can be optimized for a particular sample, and then applied consistently to multiple NMR experiments with a single command.

2.1 Loading pulse widths and power levels with **getprosol**:

Use the calibrated proton P1 value obtained from the proton experiment (protocol PRESAT_bio.nan) and note the standard power level attenuation in dB for P1 (PLW1); otherwise type calibo1p1 and wait till finished.

Then execute the getprosol command:
getprosol 1H [calibrated P1 value] [power level attenuation for P1 (PLdB1)]
e.g. getprosol 1H **9.9** -13.14.
Where for example, the calibrated **P1=9.9** at power level -13.14 dB attenuation

This also loads default ^{15}N and ^{13}C pulse widths and power levels from the PROSOL table, and are assumed to be sufficiently accurate.

go to step #2.3 If not using BioTop

2.2 Loading experimental parameters from **BioTop**:

If you previously performed parameter calibrations using the "Optimizations" tab of the BioTop GUI, or entered parameters manually in the "Optimizations" tab, you can simply

type **btprep** at the command line.

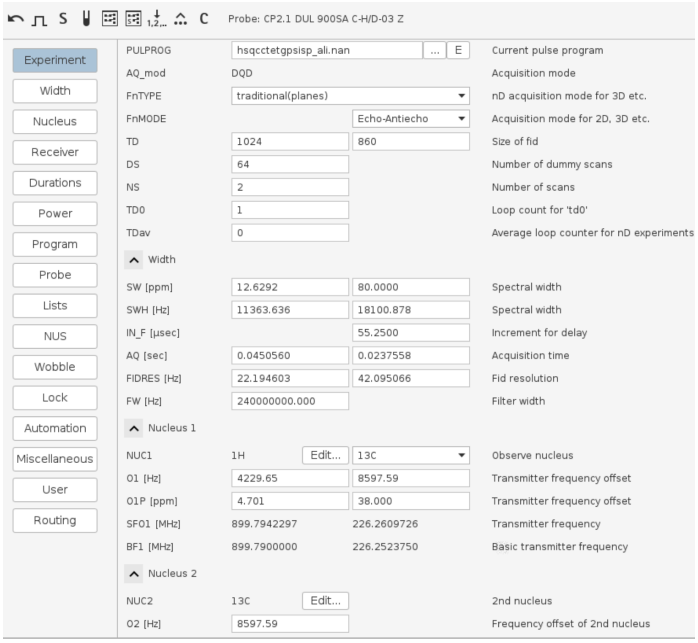
This is equivalent to calling **getprosol** with all ^{1}H, ^{13}C and ^{15}N optimized parameters followed by additional parameters loaded from "Optimizations" tab in BioTop GUI. In this particular case these parameters are based on the *bt_hsqcctetgpsisp_ali.nan.xml* description file: ^{1}H offset in Hz (**O1**), ^{1}H spectral width (**2 SW**), ^{15}N offset in ppm (**O3P**), half contant-time (CT) delay (**D23**), ^{13}C spectral width (**1 SW**), ^{13}C max acquisition time (**1 AQ**), ^{13}C offset in ppm (**O2P**), ^{13}CO offset in ppm (**CNST21**).

Inspect and adjust parameters

2.3 The default parameters from HSQC_CT_13C_ALI_xxx.par will provide an aliphatic 2D 13C CT-HSQC spectrum of a typical protein sample collected with the traditional sampling scheme (i.e. not using non-uniform sampling 'NUS'). Often the only parameters to change will be **NS = number of scans** in order to increase the signal to noise, and **1 TD** (TD in F1) that changes the number of increments (points) and hence the resolution in the ^{13}C dimension.
Check the experiment time (type **expt** or click on 'clock' icon) after any change.
These and additional parameters can be accessed and changed on the parameter windows seen below.

2.4 Select the 'Acqpars' tab to display acquisition parameters. Two display modes can be selected, the full display mode (click on the '**A**' icon or type **eda**), or pulse program-specific mode (click on the 'pulse' icon, or type **ased**). The former gives you access to all parameters and provides an overview of all spectral dimensions at once, while the latter is useful because it only displays acquisition parameters used in the pulse sequence and can be parsed sequentially as a checklist.

First examine the specific dimension parameters in Acqpars 'eda' mode (click 'A' icon). This view gives a comprehensive list of parameters covering two dimensions, F2(1H) and F1(13C) in columns.

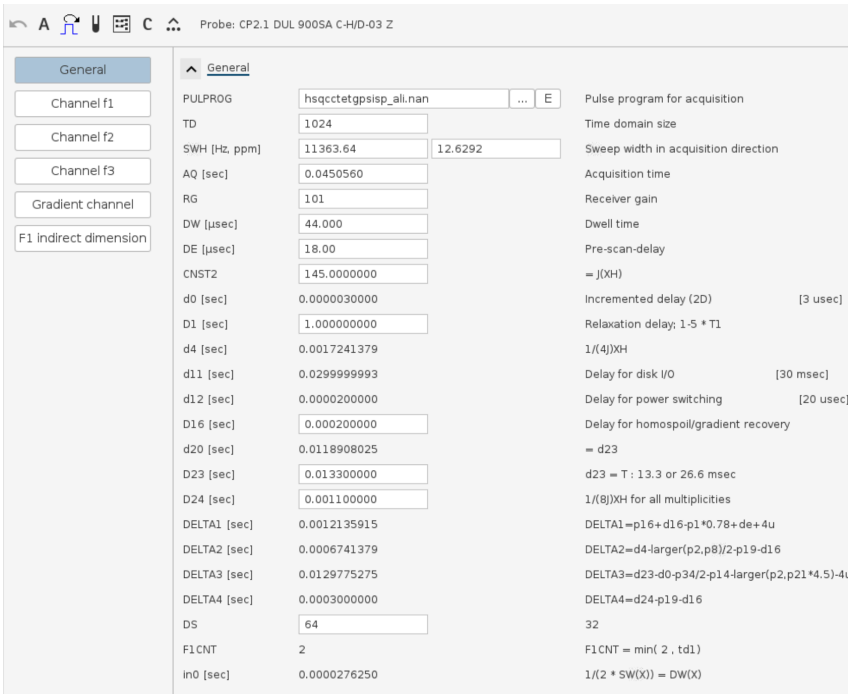


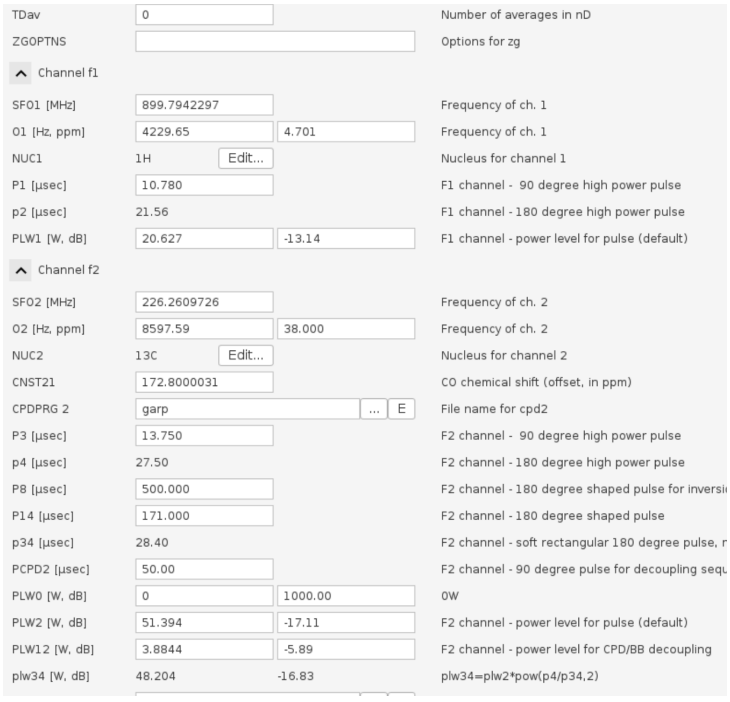
Parameters to check:

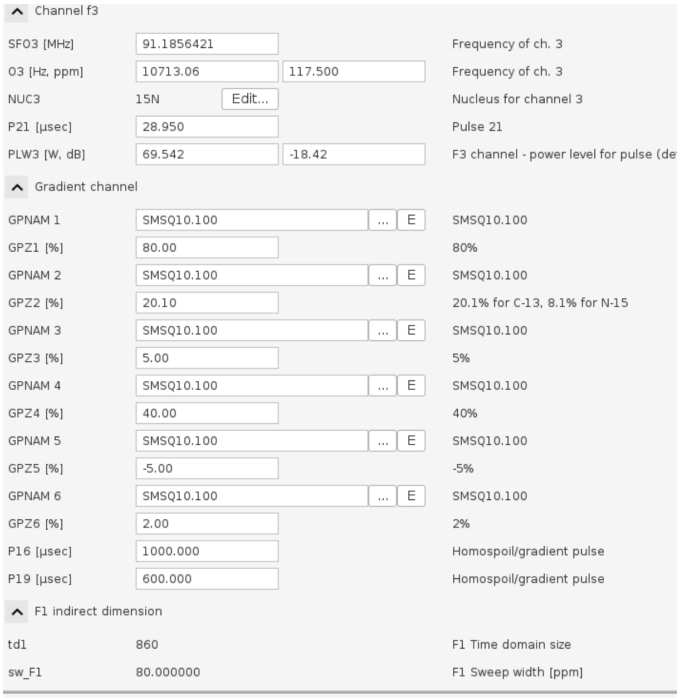
- **FnTYPE** - 'traditional planes' or 'non-uniform sampling' - see step 3 below
- **NS** - minimum 2; increase for for higher signal to noise (S/N increases as square root of NS)
- **DS** - 32-128 'dummy' scans that are not recorded; allows system to reach steady state equilibration. This is especially important for HSQC since ^{13}C decoupling during acquisition and can heat the probe and sample.
- **SW[ppm]** - F2(1H) ~12-15 ppm; F1(13C) ~80 ppm, defined in bioTop
- **O1** - ^{1}H H_2O offset in Hz (calibrated with BioTop or calibo1p1, **O1P** will be around 4.7 ppm depending on temperature)
- **O2P** - ^{13}C aliphatic offset (~38 ppm, defined in bioTop)
- **O3P** - ^{15}N offset (~115-120 ppm, defined in bioTop)
- **2 TD** - number of ^{1}H time domain real points (~1024-2048, preferably 2^{N}, keep **2 AQ** at ~50-120 ms)
- **1 TD** - number of ^{13}C time domain real points (int(2*d20/in20) for maximum signal-to-noise)
- **DIGMOD** - 'baseopt' (zero 1st order phase correction)

2.5 Then examine the parameters in the pulse program-specific '**ased**' mode (click on the 'pulse' icon). Most parameters are also accessible in the '**eda**' mode (step 2.4 above). However, the '**ased**' mode allows more convenient access to individual parameters within arrays, such as delays, pulse widths, constants, etc. It also displays parameter

values computed internally within a pulse sequence, and provides context description from the relevant pulse program comment lines.







Most of the default parameters should be appropriate, however it's useful to compare values in the fields against suggestions in the pulseprogram comments. In general, only a few may need to be changed.

- **CNST2** - effective one-bond $^{1}J_{CH}$ coupling value (≥140 Hz for aliphatic groups); used to calculate INEPT transfer delays
- **D1** - 1-2 sec
- **D23** - constant-time half-delay for ^{13}C evolution (13.3 ms)
- **D24** - refocused INEPT transfer delay (~0.0012 s). This is a compromise value to balance polarization transfer efficiency for all multiplicities (CH, CH_2, and CH_3).
- **P1** - ^{1}H 90º high power pulse (calibrated with calibo1p1 or BioTop)
- **P3** - ^{13}C 90º high power pulse (calibrated with BioTop)
- **CNST21** - ^{13}CO shift for decoupling (~173 ppm, defined in bioTop)
- **P21** - ^{15}N 90º high power pulse (calibrated with BioTop)

Configure NUS (non-uniform sampling) - optional

3 After all other acquisition parameters (especially spectral widths and time-domain points) are set, change the **FnTYPE** parameter to 'non-uniform sampling' (type 'eda' and select 'Experimental' to get correct parameter window).

Navigate down to the 'NUS' section in the 'eda' parameter window and set the desired **NusAMOUNT** [%] sampling density. For 2D ^{13}C CT-HSQC sampling density should be around 50%.

You have the option of using either the built-in sampling schedule generator in Topspin or a third-party one.

To use the built-in sampling schedule generator in TopSpin set the **NUSLIST** parameter to 'automatic'. The sampling schedule will then be generated at acquisition start, and will be purely random apart from point density weighting according to **NusJSP** and **NusT2** parameters.

A better way to generate the sampling schedule is with **nusPGSv8** AU program. This AU program uses **NusAMOUNT** and **TD** values of the current experiment to generate a random schedule with 'Poisson gap' point spacing, and offers additional options for point density weighting and sampling order. (see protocol **'**Poisson Gap NUS Acquisition Setup', and attached files **'nusPGSv8'** and **'poissonv3'**). To use this method, type **'nusPGSv8'** on the command line. You can typically accept the default values in pop-up dialog windows, since they are suitable for most applications. A schedule will be generated and will be stored to the parameter **NUSLIST**.

If **nusPGSv8** is not installed, copy the attached file 'nusPGSv8' to your user AU directory, */opt/topspin.X.X.X/exp/stan/nmr/au/src/user*, and copy the binary file 'poissonv3' to */opt/topspinX.X.X/prog/bin.*

Acquire and Process Data

4 Type '**expt**' to calculate the expected run time.

go to step #2.3 If necessary to re-adjust parameters

Type '**rga'** or click on 'Gain' in Topspin Acquire menu to execute automatic gain adjustment.
Type '**zg**' or click on 'Run' in Topspin Acquire menu to begin acquisition.

4.1 You can always check the first FID by typing '**efp**' to execute an exponential multiplied Fourier transform. It will ask for a FID #, choose the default #1. You can evaluate the 1D spectrum for amide proton signal to noise and water suppression.

To take a look at the 2D, wait for >= 16 FIDS and then click on 'Proc.Spectrum' on the Topspin Process menu. This will execute an automated processing macro.
Although the resolution will be poor, you can evaluate the signal to noise (S/N) and whether the ^{13}C offset and spectral width are acceptable.

This can be repeated at any time as additional FIDs are acquired.

Protocol references

J.Cavanaugh, W.Fairbrother, A.Palmer, N.Skelton: Protein NMR Spectroscopy: Principles and Practice. Academic Press 2006 ; Hardback ISBN: 97801216449189, eBook ISBN: 9780080471037

G.W. Vuister & A. Bax, J. Magn. Reson. 98, 428-435 (1992))
A.G. Palmer III, J. Cavanagh, P.E. Wright & M. Rance, J. Magn. Reson. 93, 151-170 (1991)