

Supplementary Data

A Routine Experimental Protocol for qHNMR Illustrated

with Taxol[⊥]

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[⊥] Dedicated to Dr. Norman R. Farnsworth on the occasion of his 77th birthday.

S1. A Cookbook Approach^a to qHNMR Providing General Guidelines for the Choice of NMR Acquisition and Processing Parameters

parameter	suggested value	comments
<i>Acquisition Parameters</i>		
acquisition time (<i>aq</i> or <i>at</i>)	2-4 s	Will vary with sample/spectral window select the short value for inverse-gated decoupled qHNMR to reduce heating effects.
relaxation delay (<i>dl</i>)	10 s	Should reflect 5 times the longest T ₁ in the sample for 90° pulses; longer delays are maintained to reduce the duty cycle of the decoupler in inverse-decoupled qHNMR
pulse width (<i>pw</i> or <i>pl</i>)	15°-45°	Ideally, use Ernst angle calculated for each sample (function of the longest T ₁ in the sample)
time domain	64 k	Should be zero-filled to at least 256 k points, but not linear predicted
spectral width (<i>sw</i>)	sample spectral window ± 3ppm on each end	Depends on the type of electronic filtering used (analog/digital) and should be pre-determined for the general use case and stored in the qHNMR parameter set.
transmitter offset	center of the ¹ H spectral width	Automatically set by spectrometer
receiver gain	closely below the highest possible setting	Automatically set by spectrometer but large values should be avoided.
number of scans/transients	128-1024	Dependant on molar concentration of the sample, desired S/N, magnetic field strength, and level of desired quantitative accuracy required
<i>Processing Parameters</i>		
window function	lb=0.1-0.3	Minimum exponential multiplication, alternative window functions may be considered, e.g., Gaussian multiplication with lb -0.3 and gb 0.05, or TRAF[S]
phasing	manual	Still the best way to do it
baseline correction	n th order polynomial	Manually optimized

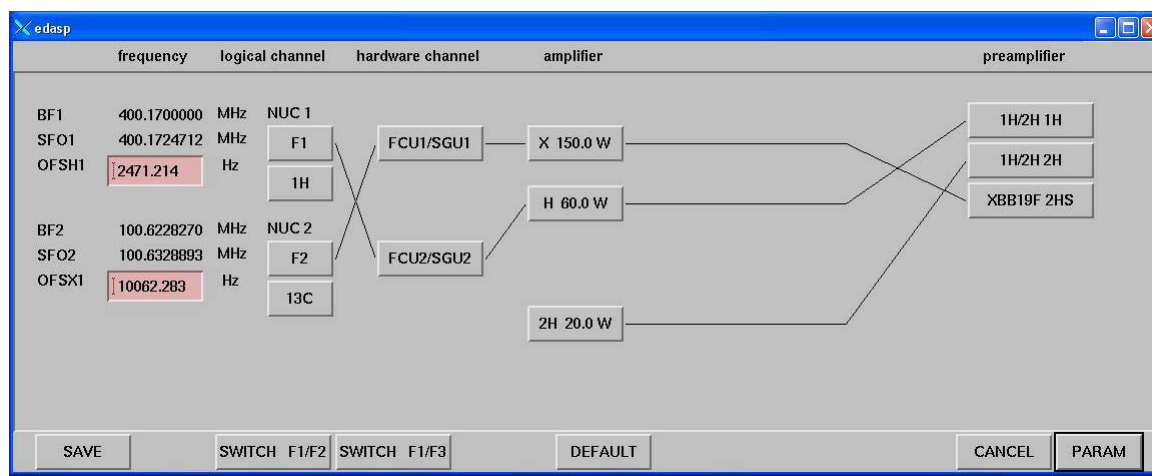
^a Updated version of Table 1 from Pauli, G. F.; Jaki, B.; Lankin, D. *J. Nat. Prod.* **2005**, 68, 133-149.

^b Typical requirement: ≥256 sans @ 400 MHz w/5mm probe for 10 mg of a 500 amu cpd.

S2. Respective Vendor Implementation of the Acquisition of Routine GARP ^{13}C -Decoupled qHNMR Spectra (Vendors in Alphabetical Order)

S2-A Routine GARP ^{13}C -Decoupled Quantitative Proton NMR (qHNMR) on Bruker NMR Spectrometers

By typing 'edasp', a display similar to that shown below will appear. The nucleus and frequency information must be modified by the user to permit ^{13}C decoupling during the acquisition. The routing panel should look like the panel shown below.



Below is the standard pulse sequence code 'zgig30' for inverse decoupling (decoupling during acquisition) using a 30° excitation pulse. It is available on instruments purchased from Bruker-Biospin. The standard sequence 'zgig', where a 90° pulse is employed, may also be used. Both may be used without modification and a parameter set using either sequence may be created by the user called 'qHNMR30_GARP_C' and 'qHNMR90_GARP_C' using these pulse sequences, respectively.

```
;zgig30
;avance-version (02/05/31)
;1D sequence with inverse gated decoupling
;using 30 degree flip angle

#include <Avance.incl>

"d11=30m"

1 ze

    d11 pl12:f2

2 30m do:f2

    d1

    p1*0.33 ph1

    go=2 ph31 cpd2:f2

    30m do:f2 mc #0 to 2 F0(zd)

exit

ph1=0 2 2 0 1 3 3 1
ph31=0 2 2 0 1 3 3 1

;p11 : f1 channel - power level for pulse (default)
;p112: f2 channel - power level for CPD/BB decoupling
;p1  : f1 channel - 90 degree high power pulse
;d1  : relaxation delay; 1-5 * T1
;d11: delay for disk I/O [30 msec]
;NS: 1 * n, total number of scans: NS * TD0
;cpd2: decoupling according to sequence defined by cpdprg2
;pcpd2: f2 channel - 90 degree pulse for decoupling sequence

;$Id: zgig30,v 1.7 2002/06/12 09:05:24 ber Exp $
```

S2-B *Routine GARP ¹³C-Decoupled Quantitative Proton NMR (qHNMR) on JEOL NMR Spectrometers*

S2-B1 *Standard 1-D Single Pulse Experiment*

```

-----
--                                     --
--               Experiment Source Code   --
--   Delta NMR Experiment & Machine Control Interface   --
--                                     --
--               Copyright (c) 2006 JEOL Ltd   --
--               All Rights Reserved         --
--                                     --
-----
-- File name : single_pulse.ex2

header
  filename => "single_pulse";
  sample_id => "";
  comment  => "single_pulse";
  process  => "process_interactive_global 'std_proton_autophase.list'";
  include  "header.include2";
end header;

instrument
  include "instrument.include2";
end instrument;

acquisition
  x_domain    => "Proton";
  x_offset    => 5[ppm];
  x_sweep     => 15[ppm];
  x_points    => 16384;
  scans      => 8;
  x_prescans  => 1;
  mod_return  => 1;
  include     "acquisition.include2";
end acquisition;

pulse
  collect COMPLEX,OBS;

  x_angle  => 45[deg], 1[deg] -> 360[deg] : 1[deg], help "flip angle";
  x_90_width => x90, help "set 90deg pulse width";
  x_atn    =? xatn, help "attenuator for x_pulse";

```

```
x_pulse  =? x_90_width * (x_angle / 90[deg]), help "observe 90[deg] pulse";

initial_wait  = 1[s];
relaxation_delay => 5[s], help "inter-pulse delay";
repetition_time =? relaxation_delay + x_acq_time, help"relaxation_delay+x_acq_time";

include "pulse.include2";

phase      = {0, 90, 270, 180, 180, 270, 90, 0};
phase_acq  = {0, 90, 270, 180, 180, 270, 90, 0};

begin
  initial_wait;
  relaxation_delay;
  x_pulse, (obs.gate, obs.phs.phase, obs.atn.x_atn);
  acq( dead_time, delay, phase_acq );
end pulse;
```

S2-B2 *Standard 1-D Single Pulse Experiment with GARP Decoupling During the Acquisition*

Differences from the code in **S2-B1** are bolded and are required for implementation of GARP decoupling during acquisition.

```

-----
--                                     --
--               Experiment Source Code   --
--      Delta NMR Experiment & Machine Control Interface  --
--                                     --
--               Copyright (c) 2006 JEOL Ltd   --
--               All Rights Reserved         --
--                                     --
-----
-- File name : single_pulse_quant.ex2

header
  filename => "single_pulse_quant";
  sample_id => "";
  comment  => "single_pulse";
  process  => "process_interactive_global 'std_proton_autophase.list'";
  include "header.include2";
end header;

instrument
  include "instrument.include2";
end instrument;

acquisition
  x_domain      => "Proton";
  x_offset      => 5[ppm];
  x_sweep       => 15[ppm];
  x_points      => 16384;
  scans         => 8;
  x_prescans    => 1;
  mod_return    => 1;
  include "acquisition.include2";
end acquisition;

pulse
  collect COMPLEX,OBS;

  x_angle  => 45[deg], 1[deg] -> 360[deg] : 1[deg], help "flip angle";

```

```

x_90_width => x90, help "set 90deg pulse width";
x_atn    => xatn, help "attenuator for x_pulse";
x_pulse  =? x_90_width * (x_angle / 90[deg]), help "observe 90[deg] pulse";

initial_wait  = 1[s];
relaxation_delay => 5[s], help "inter-pulse delay";
repetition_time =? relaxation_delay + x_acq_time, help "relaxation_delay+x_acq_time";

decoupling => TRUE, help "select TRUE or FALSE for decoupling or non_decoupling";

when decoupling do
  irr_domain => "Carbon13", help "decoupled nucleus";
  irr_offset => 100[ppm], help "decoupler offset";
  irr_pwidth => irr90_hi, help "CPD pulse width";
  irr_atn_dec => irr_atn_hi, 9->119:0.01[dB], help "attenuator setting during
acquisition";
  irr_noise => "GARP", noise_names, help "decoupler noise modulation";
end when;

include "pulse.include2";

phase    = {0, 90, 270, 180, 180, 270, 90, 0};
phase_acq = {0, 90, 270, 180, 180, 270, 90, 0};

begin
  initial_wait;
  relaxation_delay;
  x_pulse, (obs.gate, obs.phs.phase, obs.atn.x_atn);

when decoupling do
  on (irr.gate, irr.rf_mod.irr_noise, irr.atn.irr_atn_dec);
end when;
  acq( dead_time, delay, phase_acq );
when decoupling do
  off (irr.gate, irr.rf_mod.irr_noise, irr.atn.irr_atn_dec);
end when;
end pulse;

```


S2-B3 *Screen Shot of the Standard 1-D Proton NMR Experiment*

S2-B4 *Screen Shot of 1-D qHNMR Experiment Parameter Setup with Decoupling Checked and GARP Applied*



S2-C *Routine GARP ¹³C-Decoupled Quantitative Proton NMR (qHNMR) on Varian NMR Spectrometers*

The following macro, 'qhnmr', is executed to modify a standard proton observe parameter set, which uses the 's2pul' pulse sequence, for quantitative proton NMR with carbon decoupling during the acquisition. Carbon decoupling will suppress the signals arising from the carbon-13 satellites. Adjustment of the receiver gain on each sample should be performed prior to initiating data acquisition. The modification or addition of other parameters may be included in the setup macro at the users discretion.

S2-C1 *Modification of the Key Acquisition Parameters*

pw(90)	“Reads the current value of 90° pulse and sets pw = ‘pw90’” “A pulse width < 90 can also be used”
d1 = 10	“Sets the relaxation delay to a recommended value which” “includes additional delay time to limit the duty cycle of the” “decoupler to reduce or minimize the effects of sample heating”
at = 2.0	“The acquisition time can be set longer depending on the desired” “digital resolution”
fn = 262144	“Zero fill”

S2-C2 *Modification of the Decoupler Parameters for ¹³C Decoupling*

dn = 'C13'	“Set decoupler nucleus to ¹³ C”
dof = 0	“Set the decoupler offset to the center of a” “200 ppm carbon window”
dpwr = 43	“The properly calibrated value for decoupler power in dB” “should be entered here”
dmm = 'ccg'	“Set the decoupler modulation mode to GARP”
dres = 1.0	
dm = 'nny'	“Decoupling only during the acquisition time”
dmf = 36529	“The proper calibration value for the decoupler modulation” “frequency for your spectrometer / probe should be entered here”