Typical Organic Solids

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I: Measure ^{1}H 180° pulse widths.

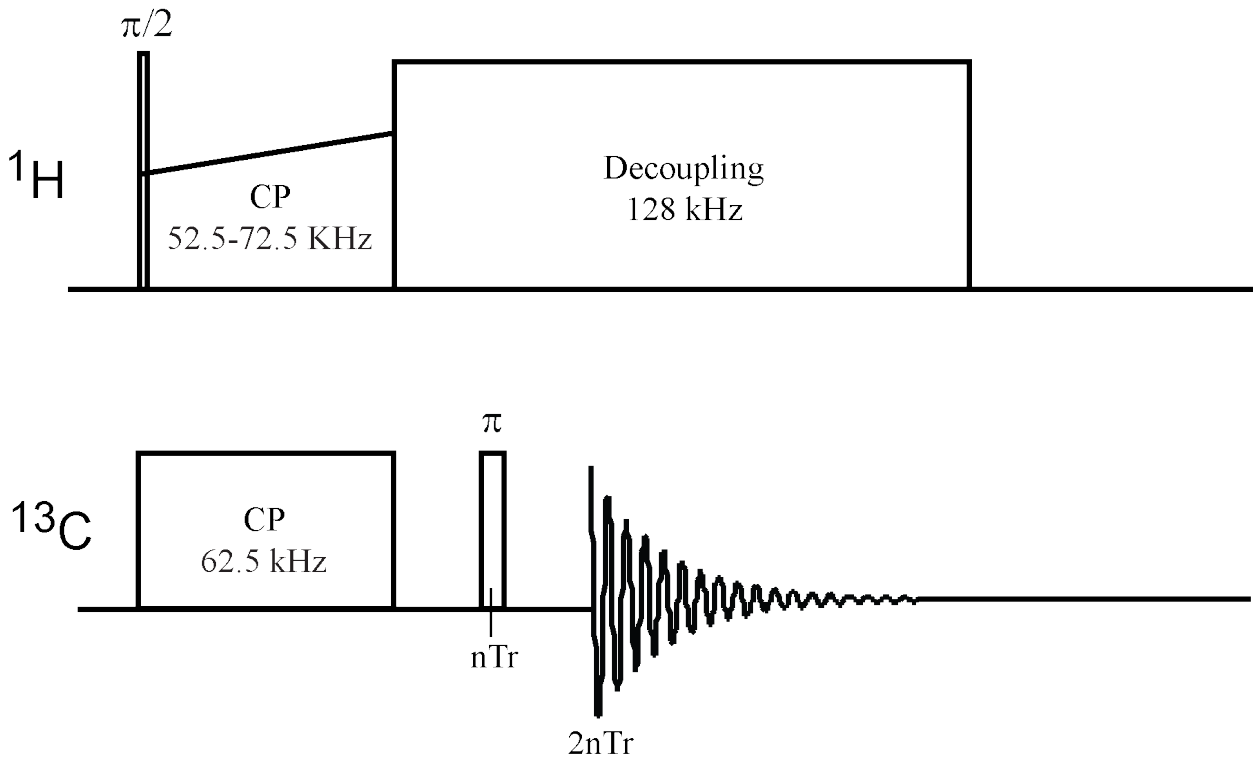


Figure 1: *^{13}C CP-Echo sequence with ramp CP used to measure ^{1}H 180° pulse by varying P3 pulse length. (See note on ramp vs square CP in Section IV)*

(1) Use standard cross polarization sequence (**Figure 2**) with ^{1}H and nuclei of interest enabled (eg. ^{13}C or ^{15}N)

(2) Set acquisition time to ~ 40ms, contact time to ~2ms

NOTE: At this point T_1 for protons is unknown, so recycle delay may not be set correctly. To minimize complications from the recycle delay set the number of scans to 1.

(3) Set ^{1}H π/2-pulse width to 1.9 μsec (or ^{1}H 90° pulse equivalent) to obtain signal.

(4) Phase spectrum using only ph0 – alternatively, put the peaks of interest on-resonance.

NOTE: Only use zero-order phase correction (ph0) as first-order phase correction assumes that lines are infinitely narrow. If a first-order phase correction is required, then there is a timing error associated with either the rotor-synchronized π–pulse or the timing of acquisition.

(5) Change the initial ^{1}H pulse width to twice the value previously measure for $\pi/2$ for adamantane and array ^{1}H power level to find power level that gives no signal. This power level can be used for both ^{1}H initial $\pi/2$ pulse and for ^{1}H decoupling.

(6) Set ^{1}H initial pulse width ½ of the value determined for the π-pulse length.

Table 1. Bruker Parameters used in CP pulse sequences

Parameter	Description	Typical Values (@10 kHz MAS)	Section
PL1	^{13}C CP Power level	Power level corresponding to a ^{13}C 180° pw = 10 μs (50kHz)	IV
PL2	^{1}H CP Power Level	Power level corresponding to a ^{1}H 180° pw = 6.5μs (77 kHz)	V
PL11	^{13}C π-pulse Power Level	Power level corresponding to a ^{13}C 180° = 4 μs (125 kHz)	IV
PL12 & PL13	^{1}H 90° and decoupling Power Level	Power level corresponding to a ^{1}H 180° = 4.0μs (125 kHz)	V
p2	^{13}C π-pulse	4.0 μsec (125 kHz)	III
p3	^{1}H 90° pulse width	2.0 μsec (125 kHz)	
p15	CP Contact Time	1.75 – 2.75 msec (optimized with CP build-up)	VII
p31	^{1}H spinal pw	^{1}H 165° at 3.7μs (125 kHz)	
D1	Recycle Delay	$5*T_1$ via ^{1}H T_1 Inversion Recovery	II
D30	Preamp delay	~ 100μs to remove receiver gate transient	
O1P	^{13}C frequency	~177 ppm (i.e. close to ^{13}C resonance)	
O2p	^{1}H offset	5 ppm	
SW	^{13}C spectral width		
cnst31	rotor period	100 μs (10kHz)	
L2	Hahn-echo time	2 rotor cycles (L2*Tr must be greater than D30)	
Aq	Acquisition time	Try to keep under 50ms to minimize probe heating and duty cycle	
cpdprg2	^{1}H decoupling	Spinal.64.13	
spnam0	ramped CP	ramp70	

II: Measure ^1H T_1

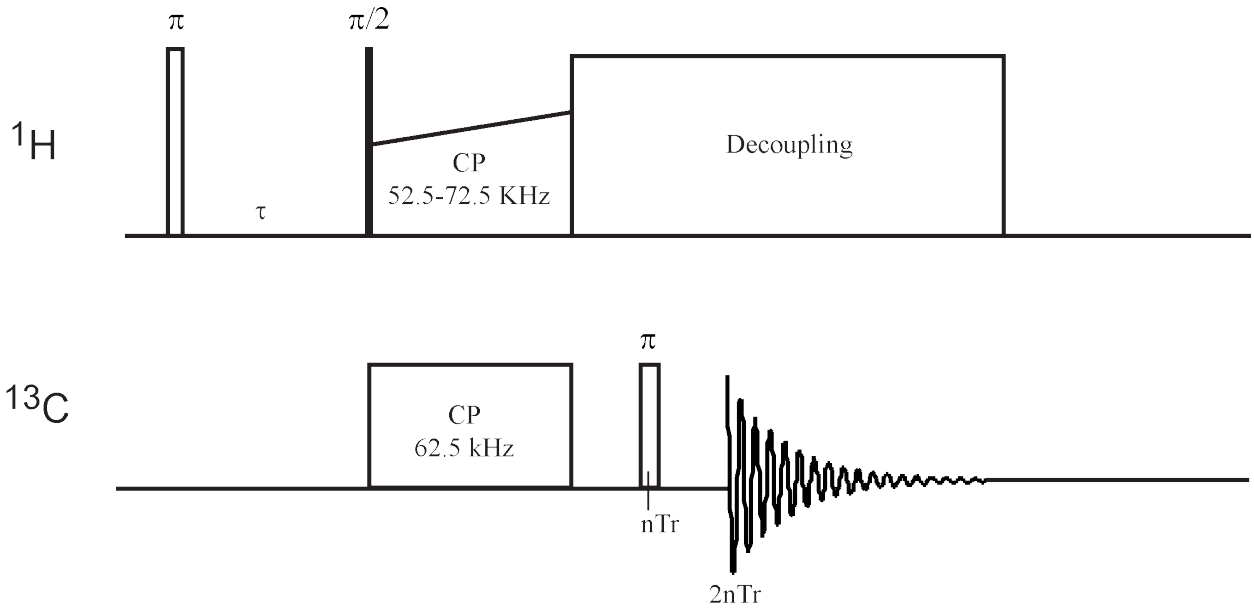


Figure 2: *T_1 inversion recovery sequence to measure τ_{null}.*

NOTE: This pulse program is a T_1 inversion recovery experiment, where we will measure τ_{null}.

(1) Add a ^1H pulse and delay (τ) before the standard cross polarization sequence (**Figure 11**) with ^1H and nuclei of interest enabled (eg. ^{13}C or ^{15}N)

(2) Set **si** to a reasonable value (e.g. 8k)

(3) Set recovery delay (τ) to 1 μs then phase a negative carbon signal.

(4) Array τ to find the minimum value of that eliminates all signal (i.e τ_{null}).

(5) Approximante T_1 as $T_1=\tau_{null}/\ln 2$.

(6) The recycle delay should be set to a value of $5*T_1$. Lower values can be used, but keep the duty cycle (Time ^1H decoupling is on/Recycle delay) to be no more than least 2% or lower.

(7) To test if T_1 is set properly take 1 scan and compare intensity with 4 scans to see if signal is 4 times greater.

NOTE: Do not use a recycle delay value less than 3 seconds. A time shorter than 3 seconds will make the duty cycle more than 2% which may damage the probe and/or amplifier (duty cycle = time ^1H pulses are on/recycle delay).

III: Measure ^{13}C 180° pulse

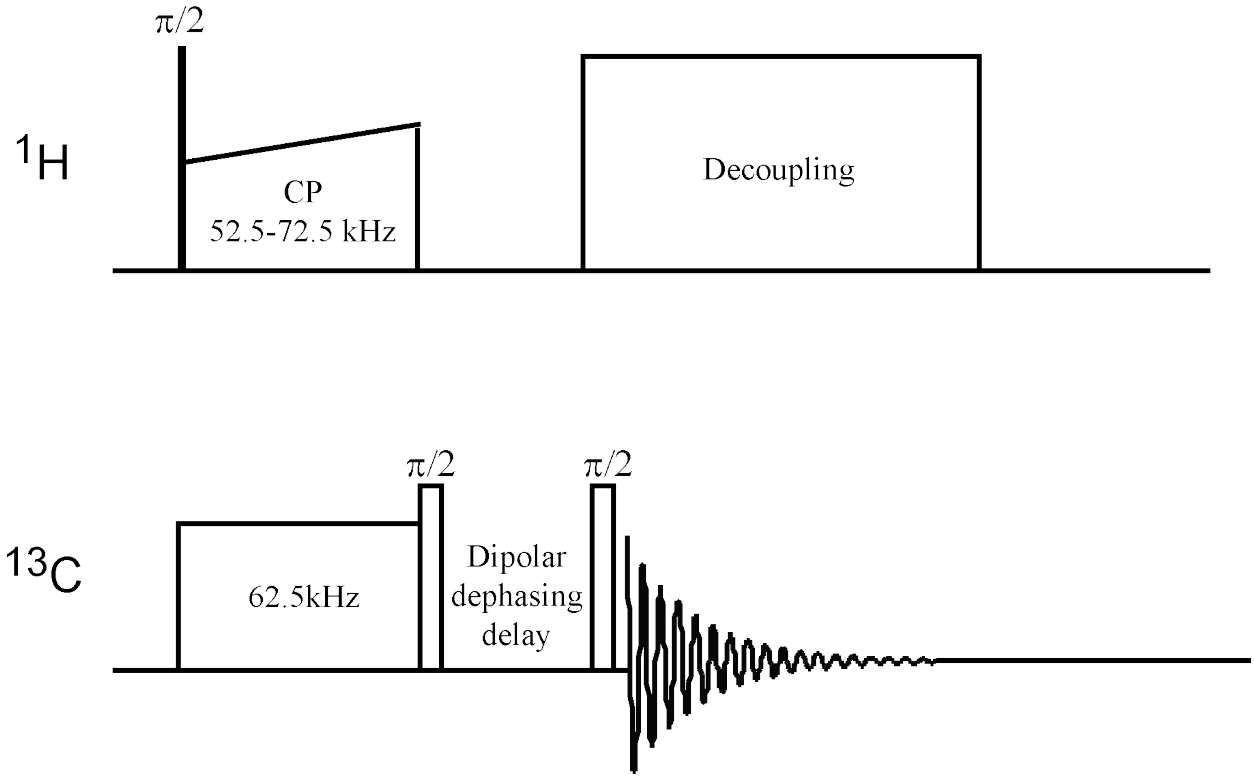


Figure 3: *Flipback-Read sequence used to measure ^{13}C 180° pulses* via *PL11.*

NOTE: The pulse power level will be optimized for 4 μs and 8 μs pulses.

(1) Use ^{13}C CP-ECHO (**Figure 4**) to put ^{13}C signal of interest on resonance. It is important to have largest peak on-resonance, otherwise this will complicate measurement of ^{13}C π-pulse

NOTE: If the signal is on resonance the FID should look like a single exponential as opposed to an oscillating FID.

(2) Add a ^{13}C pulse and delay with no ^{1}H decoupling after the standard cross polarization sequence (**Figure 3**) with ^{1}H and nuclei of interest enabled (eg. ^{13}C or ^{15}N).

(3) The first pulse after CP (Flip-Back) will be optimized to place the ^{13}C signal along the Z-axis the dipolar dephasing delay will remove any residual signal left in the x-y plane and the final ^{13}C pulse (Read) will be used to measure ^{13}C π-pulse length.

(4) Set power levels for flip-back pulse and read pulse to 0.

(5) Set the dipolar dephasing delay to 1 μs

(6) Observe signal and properly phase using only zero-order phase correction.

(7) Set the flip-back pulse width to 2.5 μs (or ½ of the π-pulse expected) and array the flip-back power level to minimize signal on resonance.

(8) Vary the dipolar dephasing delay from 1 ms-4 ms to eliminate any remaining signals.

(9) Set the read pulse power level to power level found above.

(10) Set read pulse width to 2.5 μs (or ½ of the π-pulse expected) to observe signal.

(11) Properly phase signal using only ph0

(12) Set the read pulse width to π-pulse expected.

(13) Array read pulse power level to find power that gives no signal

NOTE: Since no echo π-pulse is used before acquisition, expect the signals off-resonance to be out of phase.

IV: Measure the ^{1}H CP power-level and CP Buildup Time

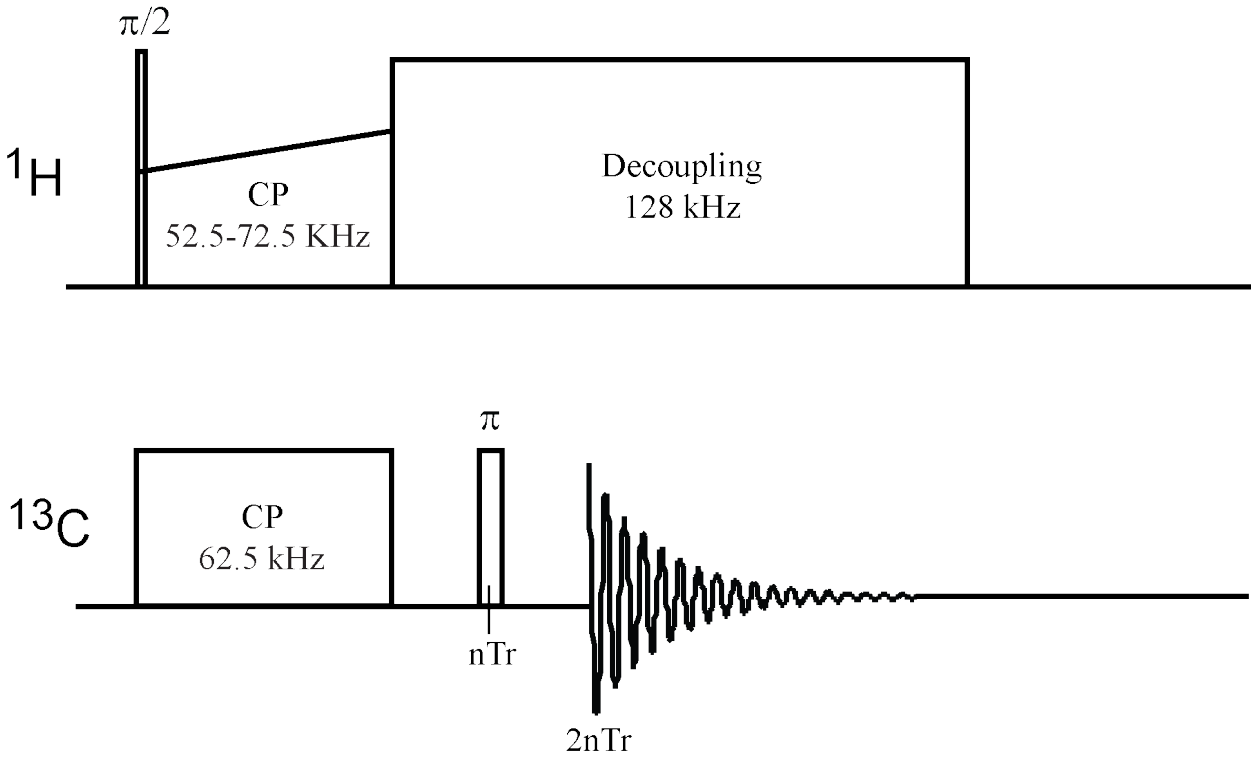


Figure 4: *CP-Echo sequence used to measure CP buildup.*

(1) Use standard cross polarization sequence (**Figure 5**) with ^{1}H and nuclei of interest enabled (eg. ^{13}C or ^{15}N)

NOTE: The ramp on the ^{1}H channel broadens the ^{1}H-^{13}C CP match condition. A good place to start is to ramp ^{1}H's from the -1 to +1 CP sideband match conditions. The optimal ramp rate will depend the strength of the ^{1}H-^{1}H and ^{13}C-^{1}H dipolar coupling, MAS rate and contact time.

(2) Set the initial ^{1}H $\pi/2$ pulse length and power to the values measured above.

(3) Set the ^{13}C power levels and pulse length for the 180° pulse and the power level measured for the 8us (62.5kHz) power levels as determined above.

NOTE: Use a ^{13}C RF field for Hartman-Hahn match to be large enough uniformly excite the entire ^{13}C CSA line shape. May have to use stronger fields at higher B_0 fields

(4) Set number of scans where signal to noise is sufficient.

(5) Array the ^{1}H CP power level

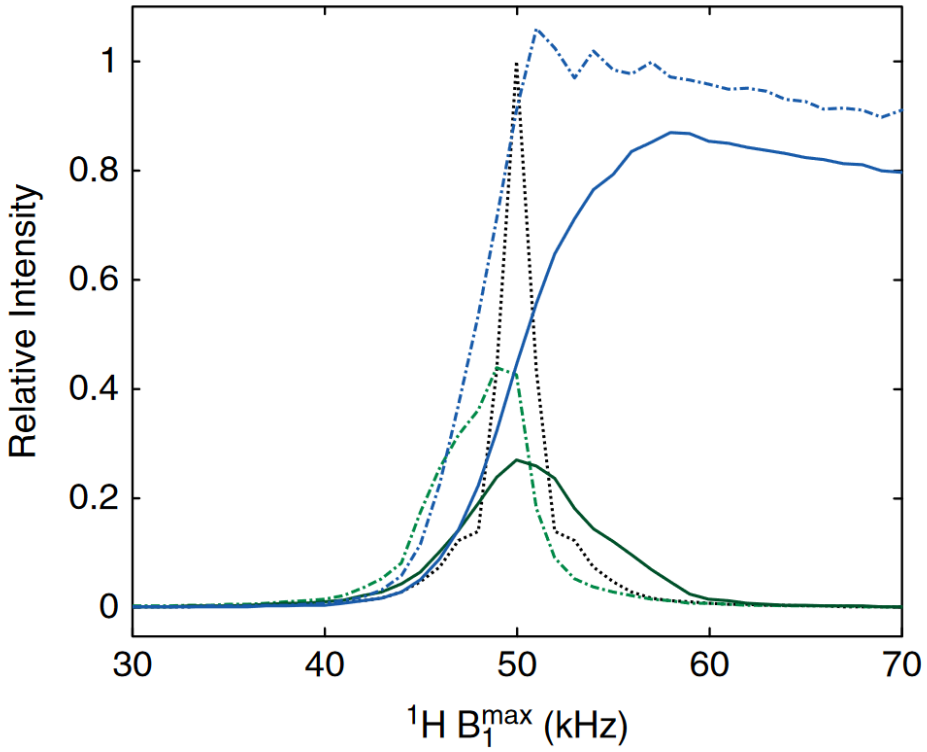
(6) Chose the power level that gives maximum signal. Use this as the power level for ^{1}H during CP.

(7) Using this power level, array the CP contact time starting at 500 μs to 3000 μs to steps of 250 μs.

(8) Choose contact time that yields maximum signal. Choose a shorter contact time over a longer time to reduce wear and tear on ^{13}C amplifier and probe.

(9) Typical ^{1}H-^{13}C contact times are ~ 2ms for lyophilized protein/peptide samples.

NOTE: Comparison of square vs ramp CP



^{1}H-^{13}C CP efficiency for a static sample using ramped and square 1H CP RF. fields with a ^{13}C RF field of 50 kHz and the ^{1}H RF field in the center of the coil as indicated. The black dotted line shows the ideal two-spin behavior with a square CP RF pulse. Blue and green plots represent behavior for ramped and square RF fields, respectively, in the case of inhomogeneous r.f. without isotropic/anisotropic chemical shift (dash-dot line) and with the ^{13}C spin having a CSA typical for a carbonyl carbon (solid line).

Ref: S. A. McNeill et al. *Magn. Reson. Chem.* **2007**; 45: S209–S220

V: Record ^{13}C CP-Echo Spectrum for Sample

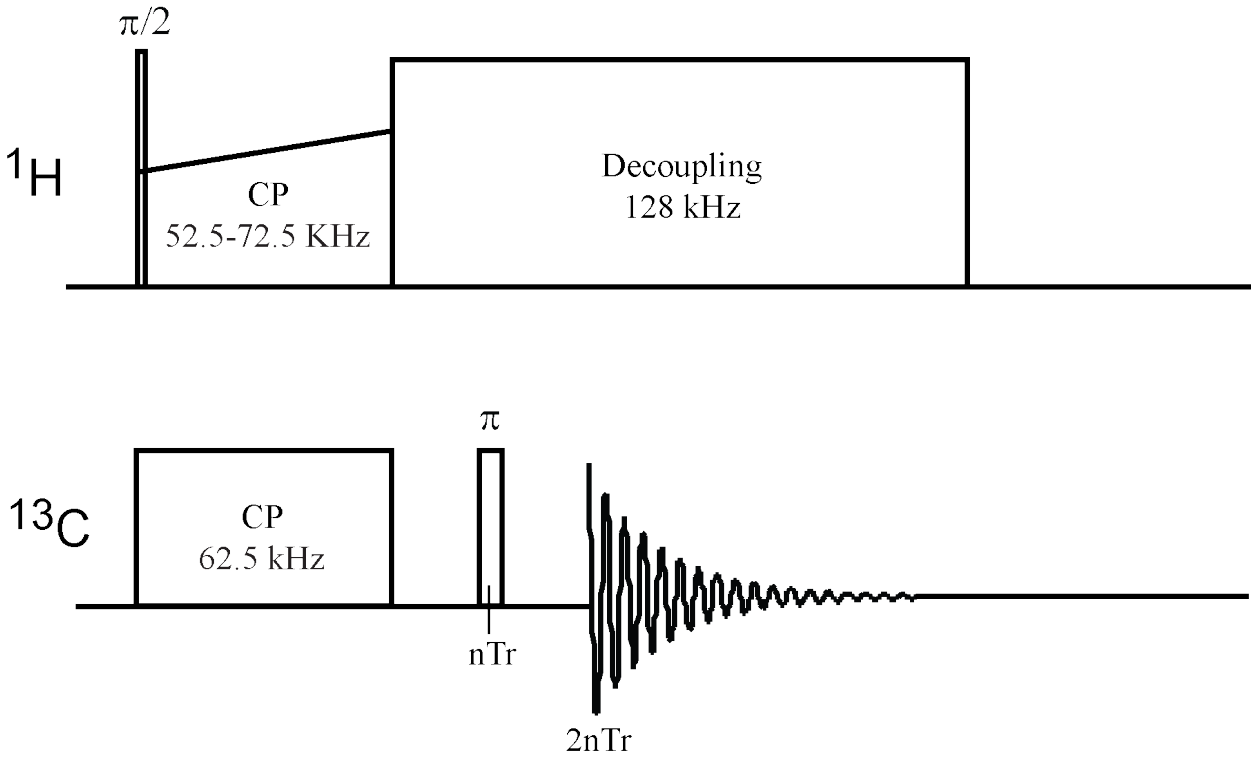


Figure 5: *CP-Echo sequence used to measure CP.*

Table 2. The following parameters must be experimentally determined for each parameter, in Fig. 6, as described in previous sections.

	Description	Value Determined By	Section Reference
1	^{1}H 90° pulse width	Measure via CP	II
2	^{1}H 90° power level	Measure via CP	II
3	CP Contact Time	CP Buildup	IV
4	^{1}H CP Power Level	From CP ^{1}H power array	IV
6	^{13}C CP Power level	Flipback-Read sequence, read pulse - 10 μs (50 kHz), 8μs (62.5kHz)	III
7	^{13}C Hahn-echo power level	Flipback-Read sequence	III
8	^{13}C Hahn-echo pulse width	4 μs (125 kHz), 5μs (100 kHz)	III
9	Recycle Delay	$5*T_1$ via T_1 Inversion Recovery	II

(1) Take 1 scan and type a verify FID takes between ½ and 2/3 of screen. If not adjust receiver gain (rg)

(2) Set **O1p** for carbon to be close to carbon of interest ~170 ppm for ^{13}CO.

NOTE: This will set resonance to be in the middle of the spectrum for a peptide sample

(3) Begin scanning and check tuning.

(4) Set number of scans to a reasonable number and begin collecting

- Review of sample heating and static vs. square CP:

McNeill, S.A., Gor'kov, P.L., Struppe, J., Brey, W.W. and Long, J.R. (2007), Optimizing ssNMR experiments for dilute proteins in heterogeneous mixtures at high magnetic fields. *Magn. Reson. Chem*., 45: S209-S220. https://doi.org/10.1002/mrc.2146

- Review on Solid-State NMR of Proteins:

Ladizhansky, V. (2014), Recent Advances in Magic-Angle Spinning Solid-State NMR of Proteins. *Isr. J. Chem*., 54: 86-103. https://doi.org/10.1002/ijch.201300096

- Review on sample heating in biological solid-state NMR:

Gor'kov, P.L., Brey, W.W. and Long, J.R. (2010). Probe Development for Biosolids NMR Spectroscopy. In *eMagRes* (eds R.K. Harris and R.L. Wasylishen). https://doi.org/10.1002/9780470034590.emrstm1149

- Review on quantitative ^{13}C solid-state NMR:

Johnson, R.L., Schmidt-Rohr, K.(2014) Quantitative solid-state ^{13}C NMR with signal enhancement by multiple cross polarization, *J. Magn. Res*., 239: 44-49. https://doi.org/10.1016/j.jmr.2013.11.009

- Guide to setting up Lee-Goldberg decoupling:

C. Coelho, J. Rocha, P.K. Madhu, L. Mafra, Practical aspects of Lee–Goldburg based CRAMPS techniques for high-resolution 1H NMR spectroscopy in solids: Implementation and applications, *J. Magn. Res*. (2008) 194: 264-282 https://doi.org/10.1016/j.jmr.2008.07.019