¹⁵N and ¹³C- SOFAST-HMQC editing enhances 3D-NOESY sensitivity in highly deuterated, selectively [¹H,¹³C]-labeled proteins.

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Supporting Information

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Variant of pulse sequence	${}^{1}\mathrm{H}_{\mathrm{N}} T_{1} (\mathrm{s})$	${}^{1}\mathrm{H}_{\mathrm{M}} T_{1} (\mathrm{s})$
A 180° hard pulse $(a1 + b1)$	0.79	0.89
A 180° hard pulse with water flip-back 90° pulses (a2 + b1)	0.62	0.84
A 180° shaped IBURP2 pulse (a3 + b1)	0.40	0.58
A 180° shaped IBURP2 pulse (a3 + b2)	0.40	0.60

Table S1. Measurement of selective T_1 recovery in ¹⁵N, [¹H, ¹³C]-Ile_{$\delta 1$}, [50%-¹H, ¹³C]-Leu_{$\delta 1, \delta 2$}, and Val_{$\gamma 1, \gamma 2$} MBP at 32 °C.*

*Inversion recovery measurements were conducted using the pulse sequence below with the a1, a2, a3, b1 and b2 variants. Faster recovery (shorter T_1) is observed when pulsing selectively on either amides or methyls ¹H's and keeping water magnetization at equilibrium.



The pulse sequence of inversion recovery for the measurement of longitudinal relaxation time T_1 . The narrow and wide bars represent 90° and 180° hard pulse. 1 ms long ¹H 90° water flip-back shaped pulse is of Sinc profile. Other ¹H shaped pulses are of 1.69 ms PC9, 1.0 ms IBURP2 and 1.15 ms REBURP (Geen and Freeman 1991), respectively. The phase cycling is: $\varphi 1 = (x, -x, -x, x)$, $\varphi_{rec} = (x, -x, -x, x)$. GARP is used for ¹⁵N or ¹³C-decoupling. The durations and strengths of the gradients are G1 = (1 ms, 15 G/cm), G2 = (1 ms, 5 G/cm). Interscan delay (d1) is 4 s, and τ is variable delay setting to 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.2, 1.6, 2.0 s, respectively, for each 1D spectrum. Different combinations are used to measure the T_1 to evaluate the impact of water exchange transfer and isolate the effect of each individual moiety on overall relaxation. Inset a2 or a3 may replace a1, and inset b2 may replace b1. When measuring ¹H_N's T_1 , ¹⁵N-decoupling with its carrier offset at 118 ppm is used, and the offsets of the shaped pulses PC9, IBURP2 and REBURP are set to 8.5 ppm. When measuring ¹H_M's T_1 , ¹³C-decoupling with its carrier offset at 17 ppm is used, and the offsets PC9, IBURP2 and REBURP are set to 0.9 ppm.

Index	SOFAST Sequence	Acquisition Dimension No. of Flip Excitation		Excitation shaped	citation shaped Refocusing	Reference Sequence	Test			
maex		F1(<i>t</i> ₁)	F2(t ₂)	F3(<i>t</i> ₃)	spectra	Angle (α)	pulse	pulse		Sample
1	<u>C_MNH_MH_N (CN-HMQC)</u>	N, C_M	H_N, H_M		1	120°	PC9_4	Reburp		a, b
					3D HM	QC-NOES	Y-HMQC			
2	$N-C_MH_M$	Ν	См	Нм	1	90-95°	PC9_4	Reburp	HMQC-NOESY-HMQC for N-CH	b
3	C_{M} -NH _N	См	N	H_{N}	1	90-95°	PC9_4	Reburp		b
4	N-NH _N	N	N	H _N	1	110°	PC9_4	Reburp	HMQC-NOESY-HMQC for N-NH	b
5	$C_M - C_M H_M$	См	См	Нм	1	110°	PC9_4	Reburp	HMQC-NOESY-HMQC for C-CH	а
6	<u>C_{Aro}-C_MH_M</u>	C _{Aro}	См	Нм	1	90-95°	PC9_4	Reburp	HSQC-NOESY-HMQC for C-CH	a,c
7	C _{Aro} -NH _N	C _{Aro}	N	$H_{\rm N}$	1	90-95°	PC9_4	Reburp		а
8	<u>NC_M-NC_MH_NH_M</u>	N, C _M	N, C _M	H_N , H_M	4	110° (90°)	Cos- PC9_4	W5'	Time Shared (TS) H-CN-H HSQC-NOESY-HSQC	b
					3D	NOESY-H	MQC			
9	<u>H_NH_{Aro}-C_MH_M</u>	H _N , H _{Aro}	См	Нм	1	90-95°	PC9_4	Reburp	NOESY-HMQC	а
10	<u>H_M-C_MH_M</u>	Нм	См	Нм	1	110°	PC9_4	Reburp	HMQC-NOESY-HMQC for H-CH	а
11	H_{M} - $C_{Aro}H_{Aro}$	Н _м	C _{Aro}	H _{Aro}	1	90-95°	PC9_4	Reburp		b
12	$H_{N}H_{Aro}-NH_{N}$	H _N , H _{Aro}	N	$H_{\rm N}$	1	110°	PC9_4	Reburp		а
13	$H_NH_M-C_MH_M$	H_N, H_M	См	Нм	2	90-95°	PC9_4	Reburp		b
14	H _N -NC _M H _N H _M	H_{N}	N, C _M	H_N, H_M	2	110° (90°)	PC9_4	W5'		b
15	$H_NH_M-NC_MH_NH_M$	$H_{N,}H_{M}$	N, C _M	H_{N},H_{M}	4	120° (90°)	cosine and sine- modulated PC9_4	W5'		b

Supplementary Table S2* List of SOFAST-NOESY sequences.

* Entries are divided into the 3D-HMQC-NOESY-HMQC and 3D NOESY-HMQC groups. The underlined entries correspond to experiments described in full in the main text. The value of flip angle in parentheses give artifact-free spectra. Experiments 8, 13, 15 are intended for ¹⁵N, methyl labeled proteins with no aromatic residues. Test samples: FliT-FliJ fusion ¹⁵N-methyl/aromatic labeled (a); ¹⁵N-methyl MBP (b). FliT-FliJ fusion double labeled (c).

[§]See Xia and coworkers for the 'Time Shared' (TS) CN-CN-H HSQC-NOESY-HSQC and H-CN-H NOESY-HSQC experiments.(Xia et al. 2003)



Figure S1. Experimental characterization of selective pulses excitation profiles. Pulse sequences of w3, w3', w5, w5' (a) and observed excitation profiles (b). In the pulse sequences w3 and w3', flip angles (α) and phases were 20.8°_x, 62.2°_x, 131.6°_x, 131.6°_{-x}, 62.2°_{-x}, and 20.8°_{-x}, respectively. In the pulse sequences w5 and w5', flip angles and phases were 7.8°_x, 18.5°_x, 37.2°_x, 70.0°_x, 134.2°_{x} , 134.2°_{-x} , 70.0°_{-x} , 37.2°_{-x} , 18.5°_{-x} , 7.8°_{-x} , respectively. τ was 188 µs (= 1/d, d is the distance in Hz between center and next null). To get the observing profiles of the PC9 4, REBURP, w3, w3', w5 and w5', a spin echo pulse sequence $(90^{\circ}_{x} + PFG + 200 \ \mu s + 180^{\circ}_{x} + PFG + 200 \ \mu s + 180^{\circ}_{x} + PFG + 200 \ \mu s + 180^{\circ}_{x} +$ observe) was used. For PC9 4 profile, the 90°_{x} was replaced with the PC9 4 shaped pulse and the 180° pulse was a hard pulse. For REBURP, w3, w3', w5, w5's profiles, the 90°_x was a hard pulse and the 180° pulse was replaced with one of the REBURP, w3, w3', w5 and w5', respectively. The bandwidths of PC9 4, REBURP and cosine-modulated PC9 4 all were 4,250 Hz (5 ppm on 850 MHz spectrometer). To get the profile of cosine-modulated PC9 4, the 2D NC-HMQC pulse sequence was used. A Bruker standard sample of 0.1 M¹³C-labeled methanol in DMSO was used for the tests. Frequency offset was swept from -7,000 to 7,000 Hz in 200 Hz steps. For each profile, a total of 71 1D spectra were acquired. Each profile pair, "w3' vs w3", "w5' vs w5", and "w5' vs w3" is color-coded and superposed. A cosine-modulated PC9(Kupce and Freeman 1994) shaped

pulse and a W5-type inversion composite pulse(Liu et al. 1998) are used to excite and refocus both amide ${}^{1}H_{N}$ and methyl ${}^{1}H_{M}$, respectively. The ${}^{15}N$ and ${}^{13}C$ spectral widths can be adjusted separately to their optimal values. W5', a modified version of W5(Liu et al. 1998) with the pulse spacing τ measured from the center of the pulse instead of the pulse edge (Supp. Fig. S1) provides more ample and uniform inversion profile. However, either 3-9-19-WATERGATE (W3)(Sklenar et al. 1993) or W5 give bandwidth sufficient to cover the narrower excitation profile of the PC9_4 selective pulse. The PC9_4 pulse with appropriate ${}^{1}H$ offset can have up to approximately 5ppm excitation bandwidth. Higher bandwidth settings reduce the water suppression effect. The cosine modulation produces a dual 5ppm excitation profile centered at the water resonance (${}^{1}H$ chemical shift ~4.7ppm).



Figure S2. Ernst angle (α pulse) optimization for the four SOFAST 3D HMQC-NOESY-HMQC variants as labeled. The optimization was conducted with d1 = 0.2 s. The optimal α value is indicated in red on each spectrum. Compatible magnetization that can transfer between scans is present only for X-XH type experiments. Low to no effect (< 5%) is observed in X-YH type experiment since the magnetization cannot be transferred from scan to scan. In that X-YH the α pulse should be set to 90-95 degrees.



Figure S3. NOE buildup curves for MBP *U*-¹⁵N, [1H, 13C]-Ile, Leu, and Val methyl sample at 32 °C (a). Comparison the of reference and SOFAST HMQC-NOESY-HMQC (main text Fig. 1d) NOE buildup curves obtained with as a series of 1D spectra acquired with 64 scans. Integrated intensities vs. mixing time were plotted at five different d1 values: 0.1, 0.2, 0.5, 1.0 and 2.0 s as labeled on the graph. Curves were fitted as described in the main text. Reference diagonal-free 3D HMQC-NOESY-HMQC ($^{15}N(F_1)$ - $^{13}C_M(F_2)$ - $^{1}H_M(F_3)$) with optimized W5' H₂O suppression (Liu et al. 1998) and flip-back pulse (b). The narrow and wide bars represent 90° and 180° hard pulse. The shaped pulse on ^{13}C channel represents a 500 µs long 180° smoothed CHIRP.(Hwang et al. 1997) The delays are: d1 = 0.2 sec, $\Delta 1 = 5.2$ ms, $\Delta 2 = 4.0$ ms, $\tau_{mix} = 0.3$ sec. The phase cycling are: $\varphi 1 = (x, -x)$, $\varphi 2 = (x, -x, -x, x)$, $\varphi 3 = 4(x)$, 4(-x), $\varphi_{rec} = (x, -x, -x, x, x, -x)$. Bruker decoupling scheme bi_garp_2pl is used. The quadrature detections in *t*1 and *t*2 dimensions are acquired via States-TPPI of $\varphi 1$ and $\varphi 2$, respectively. The durations and strengths of the gradients are G1 = (1 ms, 15 G/cm), G2 = (1 ms, 5 G/cm).



Figure S4. Ernst angle (α pulse) optimization for time-shared SOFAST 3D CN-HMQC-NOESY-CN-HMQC (upper) and for aromatic-methyl SOFAST 3D HMQC-NOESY-HMQC (lower) at fast pulsing regime (d1= 0.2 s). The optimal value for α was found to be 115 degrees, and 90 degrees respectively. Please note: the SOFAST 3D CN-HMQC-NOESY-CN-HMQC is more sensitive to the changes in α due to both amide and methyl magnetization sustaining the steady state polarization during fast pulsing. Lower effect (< 5%) is observed in X-YH-type aro-methyl experiment (b) as the magnetization cannot be transferred from scan to scan the pulse angle should be kept at 90-95°.



Figure S5. Panel a): schematic view of diagonal-free aromatic to methyl $3D^{-13}C$ -SFHMQC-NOESY-HMQC experiment. Panel b): reference $2D^{-13}C_{Aro}$ -SFHMQC spectrum (lower left spectrum in blue), reference $2D^{-13}C_M$ -SFHMQC (upper right in black), and 3D SFHMQC-NOESY-HMQC projection (lower right in red). The FliT-FliJ protein used for testing is U-[²H,¹⁵N] and contains ¹H-¹³C on all methyl residues and U-[¹³C,¹⁵N] on Phe and Tyr. The d1 was 200 ms with 32 scans per point and 40×80 points and a total experiment time of 16 h. An example NOE crosspeak between Phe H ϵ and Val C γ is marked on the spectrum and connected to the respective 2D reference dimensions.



Figure S6. Comparison of the signal strength for ¹⁵N and ¹³C 2D HSQC, HMQC, SOFAST-HMOC and TROSY experiment labeled as indicated. All the ¹³C correlation experiments use States-TPPI for phase sensitive 2D acquisition. All S/N figures indicated on the 1D strips on the left panel are derived from 2D data analysis. A sample of such analysis is shown on the right panel for selected ¹⁵N based experiments marked with an asterisk. The ratios of signal strengths are scaled separately to the ¹⁵N and ¹³C HSQC that are taken as '1'. All experiments(Schanda et al. 2005; Schulte-Herbruggen and Sorensen 2000; Solyom et al. 2013) were performed with same parameters using an ¹⁵N-methyl labeled MBP sample at 32 °C. The recovery delay (d1) was set to 0.2 s. In addition to recapitulating the findings of Schanda and coworkers(Schanda et al. 2005), a few interesting points emerge from analysis of the results. (i) The ¹⁵N-BEST-TROSY (Solyom et al. 2013) is 1.7× more sensitive with fast pulsing than the N-HSOC with WATERGATE' while the CLEAN-TROSY (Schulte-Herbruggen and Sorensen 2000) is weaker (0.9×) than the ¹⁵N-BEST-TROSY due to more complicated pulse sequence and extra pulses that are used to remove anti-TROSY components. The main benefit of ¹⁵N-TROSY is to select for the slow relaxing components and it does so at the expense of overall sensitivity, while the resolution and sensitivity can be improved by increasing both t_2 and t_1 acquisition times. Please note that the TROSY experiment uses Echo-AntiEcho (Rance-Kay) for phase sensitive acquisition and that HMQC uses States-TPPI. The former enhances sensitivity in 2D by a $\sqrt{2}$ factor.

(*ii*) Comparing the two spectra acquired with N-HMQC' and 'N-HMQC with water flip-back', the signal strength of the latter is $5.0 \times (3.0/0.6)$ that of the former. Here, the prodigious

improvement is the result of the short d1 (d1 = 0.2 s) employed for the comparison. Under these conditions, the water magnetization recovery to steady state in the 'N-HMQC' is very minimal, and the water flip-back pulse in the 'N-HMQC with water flip-back' produces the observed boost in signal. The factor is reduced from 5.0 (d1 = 0.2 s) to 2.5 (5.5/2.2) with d1 set at 1 s as shown in Supp. Fig. S4a. We also added a water flip-back pulse in the C-HMQC sequence and found that it had a significant effect on the sensitivity (1.2 to 1.4) but lower than on the amide spectrum.

(*iii*) Between 'N-HMQC with water flip-back' and SOFAST N-HMQC, the signal ratio is only changed by approximately $1.3 \times$ (from 3.0 to 3.8). These experiments should have similar water suppression effects at longer d1, and the other differences between the two are that the SOFAST N-HMQC uses 120° flip angle and the methyl's magnetization, together with the ~5% spurious protonation present in the sidechains our deuterated sample, in SOFAST N-HMQC is not perturbed resulting in a shorter T_1 relaxation time.

(*iv*) The SOFAST CN-HMQC simultaneously acquires two HMQC with a 15% S/N sacrifice for its C-HMQC region and no L-optimized effect. At fast pulsing it manages to perform better than WATERGATE sequences with flip-back mainly due to Ernst angle effect. Presently, the N-HMQC spectral region in the SOFAST CN-HMQC does not use ¹³C-decoupling pulse at the center of t_1 evolution. However, with its intended use for ¹⁵N, and selectively [¹H-¹³C]-methyl labeled samples this is no cause for concern. In these types of samples, CA and CO are not ¹³C-labeled or expected to be. When running the experiment with *U*-¹³C labeled samples, cosine-modulated SEDUCE1 can be used to decouple ¹³CA/¹³CO from ¹⁵N (Mccoy and Mueller 1992). The S/N of N-HMQC part is very close to that of simple SOFAST N-HMQC (3.7 vs 3.8) but still better than simple N-HMQC with flip-back (3.0). However, the C-HMQC part in the SOFAST CN-HMQC has an additional delay compared to a regular C-HMQC. During the first FID, the additional delay is $2(\Delta_2 - \Delta_1)$ and for the last FID, the additional delay becomes $TD\left(\frac{1}{2SW_N} - \frac{1}{2SW_C}\right) + 2(\Delta_2 - \Delta_1)$,

where *TD*, *SW_N*, *SW_C* are time domain size (2× the number of increments), spectral widths of ¹⁵N and ¹³C, respectively. Given the normal values that are used for this experiment (*TD* = 148, *SW_N* = 3101 Hz, *SW_C* = 4383 Hz, Δ_2 = 5.2 ms, Δ_1 = 4.0 ms), the additional delay goes from 2.4 ms to 9.4 ms. This compromise results in a modest 15% (=0.3/2.0) signal loss. A small penalty in the signal strength of the C-HMQC part in the SOFAST CN-HMQC is paid, but in the end, the signal for the key methyl moieties is still 1.4 times (=1.7/1.2) of that of regular C-HMQC. In 3D SOFAST NOESY experiments based on the 2D CN-HMQC, the *TD* may be set to as low as 64 points. In that case, the range of additional delay from first to last FID will be lower (2.4 ms to 5.4 ms), and the signal decay should be lower than 15%.

(v) The signal of the C-HMQC is $1.2 \times$ that of the C-HSQC. This increment is due to the shorter pulse sequence of the C-HMQC versus the C-HSQC and the methyl-TROSY effect.(Tugarinov et al. 2003) Also, SOFAST C-HMQC brings substantial improvement ($1.2 \times$ to $2.0 \times$) by means the fast recovery time between experiments due to its shorter T_1 (L-optimized effect) and by Ernst Angle optimization (120° flip angle) during fast pulsing.



Figure S7. Comparison of the signal strength for the 2D HSQC, HMQC with and without water flip back, and SOFAST N-HMQC. The shown 1D spectra are from the positive projections of full 2D spectra. The ratios of signal strengths separately are scaled to the HSQC that are taken as '1' for a) the values in parenthesis are scaled to the values in Fig. 4 for direct comparison. All experiments in panel a) or b) were performed with same parameters. In panel a), sample is an ¹⁵N-methyl labeled MBP sample at 32 °C, and the recovery delay (d1) was set to 1.0 sec. In panel b), sample is a uniformly ¹⁵N/¹³C-labeled protein (95 residues) run at 25 °C (25 mM potassium phosphate, pH 6.5, 1 mM NaN₃), and the recovery delay (d1) was set to 0.2 sec.



Figure S8. Characterization of the effect of residual $-I_z^X$ magnetization on signal intensity in X-YH-type experiments. The 'X' index refers to magnetization of protons attached to either C_M or N or C_{aro} in the case of frequency labeling of different moiety type during *first* and *second* HMQC editing in 3D HMQC-NOESY-HMQC. Panel a): pulse sequence as in Fig. 1d where 'X' = N. The shaped pulse in red labeled '¹H_N' is a test flip-down pulse that removes I_z^N magnetization recovered during mixing time τ (300 ms). Panel b): signal intensity with (black trace) or without (red trace) enhanced I_z^N recovery.

Appendix S1.

The following analysis is conducted to explain the two S/N mechanisms in the SOFAST-NOESY experiment: 1) the predominant effect of Ernst angle α in 3D SOFAST N(t1)-N(t2)H_N(t3) (or any X-XH type) and 2) the enhanced recovery mechanism in N(t1)-C_M(t2)H_M(t3) or (X-YH type) NOESY experiments.

For the ¹H-¹⁵N HMQC before NOE, the evolution of signal based on spin operator is given below:

$$kI_{z} \xrightarrow{\frac{\pi}{2}I_{x}}{-} kI_{y} \qquad (1a)$$

$$\xrightarrow{\frac{\pi}{2}2I_{z}S_{z}}{2kS_{z}I_{x}} (1b)$$

$$\xrightarrow{\frac{\pm\pi}{2}S_{x}}{+} 2kS_{y}I_{x} \qquad (1c)$$

$$\xrightarrow{\Delta\omega_{1}t_{1}S_{z}+\pi I_{x}}{+} \mp 2k[S_{y}\cos(\Delta\omega_{1}t_{1}) - S_{x}\sin(\Delta\omega_{1}t_{1})]I_{x} \qquad (1d)$$

$$\xrightarrow{\frac{\pi}{2}S_{x}}{+} 2kS_{z}\cos(\Delta\omega_{1}t_{1}) - S_{x}\sin(\Delta\omega_{1}t_{1})]I_{x} \qquad (1d)$$

$$\xrightarrow{\frac{\pi}{2}I_{z}S_{z}}{+} \mp 2k[S_{z}\cos(\Delta\omega_{1}t_{1}) - S_{x}\sin(\Delta\omega_{1}t_{1})]I_{x} \qquad (1e)$$

$$\xrightarrow{\frac{\pi}{2}I_{z}S_{z}}{+} \mp k(2I_{z})^{2}I_{y}\cos(\Delta\omega_{1}t_{1}) \pm 2kS_{x}I_{x}\sin(\Delta\omega_{1}t_{1}) \qquad (1f)$$

$$\xrightarrow{\frac{\pi}{2}I_{x}}{+} \mp kI_{z}\cos(\Delta\omega_{1}t_{1}) \pm 2kS_{x}I_{x}\sin(\Delta\omega_{1}t_{1}) \qquad (1g)$$

$$\xrightarrow{gradient PFG during \tau}{+} \mp kI_{z}\cos(\Delta\omega_{1}t_{1}) \qquad (1h)$$

In the above derivations, ¹H chemical shift is not included because ¹H π pulse in the middle of the HMQC refocuses the ¹H chemical shift evolution. After the initial $\frac{\pi}{2}$ (90°) pulse, ¹H polarization is changed to ¹H coherence I_y (eq. 1a). The scalar J coupling ${}^1J_{NH}(\pi^{-1}J_{NH}\Delta_1 = \frac{\pi}{2})$ during the first Δ_1 period changes the ¹H's coherence I_y into antiphase coherence $S_z I_x$ (eq. 1b). The first ¹⁵N $\frac{\pi}{2}$ pulse of phase $\pm x$ changes the antiphase coherence into multiple quantum (zero and double quantum) coherence $(S_{\nu}I_{x})$ (eq. 1c). Please note that different phase $(\varphi_{1} = \pm x)$ corresponds to the different sign (\mp) of signal. During evolution t_1 period, the ${}^1J_{\rm NH}$ coupling is refocused by the ${}^1H \pi$ pulse, and ¹⁵N chemical shift and the ¹H π pulse are applied so that the coherence is frequency-labeled (eq. 1d). The second ¹⁵N $\frac{\pi}{2}$ pulse changes the first term of multiple quantum coherence $S_y I_x$ back into an antiphase coherence $S_z I_x$ and leaves the second term $S_x I_x$ unchanged (eq. 1e). The scalar J coupling ${}^{1}J_{\rm NH}$ during the second Δ_{1} period changes the antiphase coherence $S_{z}I_{x}$ back into ${}^{1}{\rm H}$ coherence I_y and leaves the second term still $S_x I_x$ still unchanged (eq. 1f). Here please note that $(2I_z)^2 = 1$. Additionally, I_zS_z and I_xS_x commute ($[I_zS_z, I_xS_x] = 0$), i.e. ¹ J_{NH} coupling does not affect multiple quantum coherence, therefore the second term $S_x I_x$ is not changed. The second ¹H $\frac{\pi}{2}$ pulse changes the ¹H coherence I_y back into ¹H I_z polarization and leaves the second term unchanged (eq. 1g). Finally gradient during mixing time τ destroys the multiple quantum $S_x I_x$.

Appendix S2. Pulse program for 2D C_MNH_MH_N SFHMQC (Bruker Topspin ver. 2.1 to 3.5).

;stCNhmqcgpph19: sotast simultaneous 2D 15N/13C HMQC for HN and methyl ;Youlin Xia on 05/05/2016 ;\$CLASS=HighRes ; \$DIM=3D ; \$TYPE= ; \$SUBTYPE: : \$COMMENT= prosol relations=<triple> #include <Avance.incl> #include <Delay.incl>
#include <Grad.incl> "p4=p3*2" "p19=300" "p22=p21*2" "d11=30m" "d13=4u" "d14=4.0m" ;for 13C "d15=5.2m" ;for 15N "d0=0u" "d10=0u" "in0 =inf1/2" "in10=1s/(2*cnst8)" ;cnst8 must be less than swC ;cnst8 = 30 * sfo3, 30 ppm of spectral width for 15N "l1 = (d14-(1-0.638)*(p21-p3) -p19 -d16 -14u)*2/(in10-in0)+1" :TD(F1) <= 11 "l2 = l1" "cnst2=5.0" "p41=7.2/(cnst2*bf1/1000000)" /* PC9 pulse length */ "spw3l=4+plw1*(pow((cnst3/90.0)*(p1/p41)/0.125,2))" /* PC9 power level */ ;*sp3l=p1-20*log(2*(p1/p41)/0.125)/log(10)" /* PC9 power level */ "spoff3l=0" /* PC9 offset */ "DELTA=p4" 1 d11 ze 2 d11 do:f2 do:f3 3 d1 pl0:f1 pl12:f2 pl3:f3 BLKGRAD 10u UNBLKGRAD "d32 = d0*2 - p3*0.637*2" "if (d32 < 0) { d32 = 0; }" "d33 = d10*2 - p21*0.637*2" "if (d33 < 0) { d33 = 0; }" "d34 = d15-d14 + 0.5*(p22-p4+d33-d32)" :d34 > 0.5*p41 u34 = 013-014 + 0.34(D22-p4+035-032) , u34 > 0.38 "435 = 614-p19-d16-14" "437 = 614-p19-d16-14" "437 = 615 - 634 - p19 - 616 - 144" "if (d32 == 0) { d37-d36-p21; d38=d37-p22-p4;}" ;1st point ;d37 should be > 0 (p21 ph2):f3 p16:gp1 4u cpd2:f2 (p41:sp31 ph1):f1 d35 4u do:f2 10... p19:gp2 d16 pl1:f1 pl2:f2 if "d32 > 0" (center (p1*0.231 ph7 d19*2 p1*0.692 ph7 d19*2 p1*1.462 ph7 d19*2 p1*1.462 ph8 d19*2 p1*0.692 ph8 d19*2 p1*0.231 ph8);f1 (d36 p3 ph5 d32 p3 ph1 d36);f2 (d37 p21 ph5 d33 p21 ph8 d37);f3) else (center (p1*0.231 ph7 d19*2 p1*0.692 ph7 d19*2 p1*1.462 ph7 d19*2 p1*1.462 ph8 d19*2 p1*0.692 ph8 d19*2 p1*0.231 ph8):f1 (d36 p3 ph5 d32 p3 ph1 d36):f2 (d37 p21 ph5 DELTA p22 ph2 DELTA p21 ph8 d38):f3) p19:gp2 d16 10u pl12:f2 pl16:f3 4u cpd2:f2 d34 qo=2 ph31 cpd3:f3 d11 do:f2 do:f3 mc #0 to 2 F1PH(ip5, id0 & id10) 4u BLKGRAD exit ph1 =0 ph2 =1 ph5 =0 2 ph7 =0 ph8 =2

ph31=0 2

;pl1 : f1 channel - power level for pulse (default) plii: f2 channel - power level for pulse (default)
plii: f3 channel - power level for CPD/88 decoupling
p31: f1 channel - power level for Shaded 90 degree pulse
spnmm31: PC9_4_90.1000 with a consin-modulation ;spnam31: PC_4_00.1000 with a consin-modulati [p1 : f1 channel - 90 degree high power pulse ;p2 : f1 channel - 180 degree high power pulse ;p3 : f2 channel - 180 degree high power pulse ;p4 : f2 channel - 180 degree high power pulse ;p16: homospoil/gradient pulse [1 msec] ;pls: homospoil/gradient pulse ;pls: homospoil/gradient pulse ;p41: f1 channel - 90 degree shaped pulse ;d0 : incremented delay (F1 in 30) ;d1 : relaxation delay; 1-5 * T1 ;d2 : 1/((22)XH) ;d2 : incrementation delay; 1-5 * T1 [0.3 msec] [3 usec] 122 : j/(LJ)AH) (38 : mixing time ;d1: delay for disk I/O ;d1: short delay ;d16: delay for homospoil/gradient recovery ;d17: fixed delay [30 msec] [4 usec] ;dl8: run time delay given by the pulse sequence ;dl9: delay for binomial water suppression ; dl9= (l/(z+d)), d = distance of next null (in Hz) ;d32: run time delay given by the pulse sequence 1327 inu time delay given by the pulse sequence (337 in utime delay given by the pulse sequence (347 in utime delay given by the pulse sequence (358 in utime delay given by the pulse sequence (357 in utime delay given by the pulse sequence (357 in utime delay given by the pulse sequence (358 in utime delay given by the pulse sequence (358 in utime delay given by the pulse sequence (358 in utime delay given by the pulse sequence) ;cnst3:flipping angle [~120] ;cnst8:ISM spectral width in Hz [=36*sfo3] ;inf1: 1/SW(H) = 2 * DW(H) ;in0: 1/(2 * SW(H)) = DW(H) ;in20: 1s/(2 * cnst1) = DW(H) ;in/20: 1s/(2 * cnstl) = DW(H) ;ll: maximum TD1 value allowable ;NS: 2 * n ;DS: 2*n ;td1: number of experiments in F1 ;FnMODE: States-TPPI (or TPPI) in F1 ;rpd2: decoupling according to sequence defined by cpdprg2 ;pcpd2: f2 channel – 90 degree pulse for decoupling sequence ;cpdprg2: f2 channel [bi_garp_2p1] ;cpd3: decoupling according to sequence defined by cpdprg3 ;pcpd3: f3 channel - 90 degree pulse for decoupling sequence ;cpdprg3: f3 channel [bi_garp_2pl.2] ;for z-only gradients:

;gpz1: 20% ;gpz2: 60%

;use gradient files: ;gpnam1: SMSQ10.100 ;gpnam2: SMSQ10.50

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	Wed Jul 20 15:21:34 CDT 2016	p4 [µsec]	22.40	
		Channel fl		
Pulseprogram param	eters of dataset:	SFO1 [MHz]	850.3039930	
C:/NMR/sofast/24/pdata/1		01 [Hz, ppm]	3993.01	4.696
		NUC1	1H	
General		Pl [µsec]	10.50	
PULPROG	sfCNhmqcgpph19	p41 [µsec]	1693.52	
TD	1356	PLW0 [W, -dBW]	0	1000.00
SWH [Hz, ppm]	13586.96 15.9789	PLW1 [W, -dBW]	11.995	-10.79
AQ [sec]	0.0499008	SPNAM 31	Pc9 4 90.pw1693	.5us.cos3230Hz
RG	128	SPOAL31	0.500	
DW [µsec]	36.800	spoffs31 [Hz]	0	
DE [µsec]	25.00	spw31 [W, -dBW]	0.20985	6.78
ChSt2	5.000000	Channel f2		
CNST3	2101 8000488	SFO2 [MHz]	213.8116328	
do [aca]	5101.8000488	02 [Hz nnm]	3527 83	16 500
D1 [sec]	0 2000000	NUC2	130	10.000
	0	CDDDDC 2	hi garn 2nl	
dll [sec]	0.0300000	D2 [usod]	11 20	
dll [sec]	0.0000400	F5 [µsec]	50.00	
dl4 [sec]	0.00400000	PCPD2 [µsec]	50.00	
d14 [sec]	0.00520000	PLW2 [W, -dBW]	180	-22.55
D16 [sec]	0.00020000	PLW12 [W, -dBW]	9.0317	-9.56
D19 [sec]	0.00007400	PLW30 [W, -dBW]	9.0317	-9.56
D32 [sec]	0	PLW31 [W, -dBW]	18.021	-12.56
D33 [sec]	0	Channel f3		
D34 [sec]	0	SFO3 [MHz]	86.1703179	
D35 [sec]	0	O3 [Hz, ppm]	10166.90	118.000
D36 [sec]	0	NUC3	15N	
D37 [sec]	0	CPDPRG 3	bi_garp_2pl.2	
D38 [sec]	0	P21 [µsec]	38.00	
DELTA [sec]	0.00002240	p22 [µsec]	76.00	
DS	16	PCPD3 [µsec]	240.00	
in0 [sec]	0.00011410	PLW3 [W, -dBW]	295	-24.70
in10 [sec]	0.00016120	PLW16 [W, -dBW]	7.3955	-8.69
INF1 [µsec]	228.20	PLW32 [W, -dBW]	7.3955	-8.69
11	149	PLW33 [WdBW]	14.756	-11.69
12	149	Gradient channel		
NS	4	GPNAM 1	SMSQ10.100	

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GPNAM 2	SMSQ10.50
GPZ1 [%]	20.00
GPZ2 [%]	60.00
P16 [µsec]	1000.00
p19 [µsec]	300.00

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Appendix S4. Pulse program for 3D C_MN-C_MNH_MH_N SFHMQC-NOESY-HMQC.

;stCNhmqcnoeCNhmqc3d.NLm-NLmHnHm: simultaneous 3D CNhmqc-noesy-CNhmqc tor NLm(F1)-NLm(F2)HnHm(F3) ;YOulin Xia on 05/05/2016 "d24 = d15-d14 + 0.5*(p22-p4+d23-d22)" "d25 = d24-p41*0.5" ;d24 > 0.5*p41 "d25 = d24-p94;*0.5" "d26 = d14-p19-d15-8u" "d27 = d15 -d24 - p19- d16 -8u" "if (d22 === 0) { d27=d26-p21; d28=d27-p22-p4;}" ;1st point ;d27 should be > 0 /* /* cnst8: =36*sfo3. cnst8 (for 15N) TD(F2) <= L1 :for t2 dimension "d32 = d20*2 - p3*0.637*2" "if (d32 < 0) { d32 = 0; }" "d33 = d30*2 - p21*0.637*2" "if (d33 < 0) { d33 = 0; }" use "split ipap 2" to split the data, enter 1 for "scaling factor for AP spectrum", and enter 0 for "splitting in F1(0) or F2(1)" two sets of 3D data are acquired, one is for $N(\mbox{tl})$ + $C(\mbox{tl})$ and the other is for $N(\mbox{tl})$ – $C(\mbox{tl})$ four sub 3D spectra will be obtained: "d34 = d15-d14 + 0.5*(p22-p4+d33-d32)" ;d34 > 0.5*p41 "d34 = d15-d14 + 0.5*() "d35 = d34-p41*0.5" "d36 = d14-p19-d16-8u" 1: Cm(t1) NOE N(t2)-Hn(t3) 2: Cm(t1) NOE Cm(t2)-Hm(t3) 3: N(t1) NOE N(t2)-Hn(t3) 4: N(t1) NOE Cm(t2)-Hm(t3) "d37 = d15 -d34 - p19- d16 -8u" "if (d32 == 0) { d37=d36-p21; d38=d37-p22-p4;}" ;1st point ;d37 should be > 0 (p21 ph2):f3 p16:gp1 setting zgoptns -Dhard will use hard 90 degree pulse. 200u */ ;1st CNHMQC 4u cpds2:f2 #ifdef hard ;\$CLASS=HighRes 4u pl0:f1 ;\$DIM=3D (p11:sp1 ph8):f1 ; \$TYPE= ; \$SUBTYPE= ; \$COMMENT= 4u pl1:f1 (p1 ph1):f1 prosol relations=<triple> d24 #else 4u pl0:f1 (p41:sp29 ph1):f1 #include <Avance.incl> #include <Delay.incl>
#include <Grad.incl> d25 #endif 4u do:f2 "p4=p3*2" "p19=500" "p22=p21*2" 4u "d11=30m" "d13=4u" p19:gp2 #ifdef hard d16 pl2:f2 "d14=4.0m" ;for 13C "d15=5.2m" ;for 15N "d15=5.2m" #else d16 pl1:f1 pl2:f2 "d0=0u" "d10=0u" #endif "in0 =inf1/2" "in10=1s/(2*cnst8)" ;cnst8 must be less than swC ;cnst8 = 30 * sfo3, 30 ppm of spectral width for 15N if "d22 > 0" (center (p1*0.231 ph7 d19*2 p1*0.692 ph7 d19*2 p1*1.462 ph7 d19*2 p1*1.462 ph8 d19*2 p1*0.692 ph8 "d20=0u" d19*2 p1*0.231 ph8):f1 (d26 p3 ph3 d22 p3 ph4 d26):f2 (d27 p21 ph3 d23 p21 ph6 d27):f3) "d30=0u" "in20 =inf2/2" "in30=1s/(2*cnst8)" } else ;cnst8 must be less than swC ;cnst8 = 30 * sfo3, 30 ppm of spectral width for 15N (center (p1*0.231 ph7 d19*2 p1*0.692 ph7 d19*2 p1*1.462 ph7 d19*2 p1*1.462 ph8 d19*2 p1*0.692 ph8 "l1 = (d14-(1-0.638)*(p21-p3) -p19 -d16 -8u)*2/(in10-in0)+1" ;TD(F1) <= 2*L1, TD(F2) <= L1 d19*2 p1*0.231 ph8):f1 (d26 p3 ph3 d22 p3 ph4 d26):f2 (d27 p21 ph3 DELTA p22 ph2 DELTA p21 ph6 d28):f3) "12 = 11' "cnst2=5.0" p19:gp2 "cnst3=90" d16 "p41=7.2/(cnst2*bf1/1000000)" /* PC9 pulse length */ 4u pl12:f2 "spu29=4*plu1*(pow((p1/p41)/0.125,2))" /* PC9 power level */ ;"sp29=p11=20*log(2*(p1/p41)/0.125)/log(10)" /* PC9 power level */ "sp0ff29=0" /* PC9 offset */ #ifdef hard 4u cpds2:f2 d24 (p1 ph1):f1 "spw31=4*plw1*(pow((cnst3/90.0)*(pl/p41)/0.125,2))" /* PC9 power level */ ;"sp31=pl1-20*log((cnst3*2/90.0)*(p1/p41)/0.125)/log(10)" /* PC9 power level */ "spoff31=0" /* PC9 offset */ 4u pl0:f1 (p11:sp1 ph8):f1 4u #else 4u pl0:f1 cpds2:f2 d25 /* 15N CPD power level */ (p41:sp29 ph1):f1 #endif 4u do:f2 "DELTA=p4" "TAU=d8-p21-p16*3-0.5m-10u" ;NOESY "l0=0" TAU BLKGRAD 10u UNBLKGRAD aqseq 312 (p21 ph1):f3 p16*3:gp3 1 d11 ze 2 d11 do:f2 do:f3 0.5m 3 20u pl12:f2 pl3:f3 BLKGRAD :2nd CNHMOC d1 4u cpd2:f2 #ifdef hard 4u pl0:f1 if "10 % 2 == 0 && nsdone % ns == 0" . грб (p11:sp1 ph8):f1 if "l0 % 2 == 1 && nsdone % ns == 0" 4u pl1:f1 ip6*2 (p1 ph1):f1 d34 #else (p41:sp31 ph1):f1 d35 #endif 10u UNBLKGRAD ;for t1 dimension "d22 = d0*2 - p3*0.637*2" 4u do:f2 "if (d22 < 0) { d22 = 0; }" "d23 = d10*2 - p21*0.637*2" "if (d23 < 0) { d23 = 0; }"

Appendix S4. (continued)

```
4u
p19:gp2
   #ifdef hard
d16 pl2:f2
   #else
         d16 pl1:f1 pl2:f2
   #endif
         if "d32 > 0"
           (center (p1*0.231 ph7 d19*2 p1*0.692 ph7 d19*2 p1*1.462 ph7 d19*2 p1*1.462 ph8 d19*2 p1*0.692 ph8
   d19*2 p1*0.231 ph8):f1 (d36 p3 ph5 d32 p3 ph1 d36):f2 (d37 p21 ph5 d33 p21 ph9 d37):f3)
           else
            .
(center (p1*0.231 ph7 d19*2 p1*0.692 ph7 d19*2 p1*1.462 ph7 d19*2 p1*1.462 ph8 d19*2 p1*0.692 ph8
   d19*2 p1*0.231 ph8):f1 (d36 p3 ph5 d32 p3 ph1 d36):f2 (d37 p21 ph5 DELTA p22 ph2 DELTA p21 ph9 d38):f3)
           p19:gp2
           d16
           4u pl12:f2 pl16:f3
           4u cpd2:f2
           d34
           qo=2 ph31 cpd3:f3
           d11 do:f2 do:f3 mc #0 to 2
;for topspin2.0
                      ;F1I(iu0, 2)
;F1PH(ip3, id0 & id10)
                      ;F2PH(rd0 & rd10 & rp3 & ip5, id20 & id30)
                 ;for topspin3.0
                     FII(iu0, 2)
FIPH(calph(ph3, +90), caldel(d0, +in0) & caldel(d10, +in10))
F2PH(calph(ph5, +90), caldel(d20, +in20) & caldel(d30, +in30))
    4u BLKGRAD
   exit
   ph1 =0
ph2 =1
  ph2 =1
ph3 =0 0 2 2
ph4 =0 0 0 0 2 2 2 2 2
ph6 =0 0 0 0 2 2 2 2 2
ph5 =0 2
ph7 =0
    nh8 =2
   pn8 =2
#ifdef hard
ph9 =0
#else
   ph9 =2
    .
#endif
   ph31=0 2 2 0 2 0 0 2
;pl1: f1 channel - power level for pulse (default)
;pl12: f2 channel - power level for pulse (default)
;pl16: f3 channel - power level for cPD/B8 decoupling
;sp32: f1 channel - power level for shaped 90 degree pulse
;spnam29: PC9_4_90.1000 with a consin-modulation
;pl : f1 channel - 90 degree high power pulse
;p32: f1 channel - 90 degree high power pulse
;p32: f1 channel - 90 degree high power pulse
;p32: f1 channel - 90 degree high power pulse
;p32: f1 channel - 90 degree high power pulse
;p32: f1 channel - 90 degree high power pulse
;p32: f1 channel - 180 degree high power pulse
;p14: f2 channel - 90 degree high power pulse
;p14: f2 channel - 90 degree high power pulse
;p14: f1 channel - 90 degree shaph power pulse
;p14: f1 channel - 90 degree shaph power pulse
;p14: f1 channel - 90 degree shaph outse
;p16: homospoil/gradient pulse
;p16: homospoil/gradient pulse
;p16: homospoil/gradient pulse
;p17: f1 channel - 90 degree shaph outse
;p18: f1 channel - 90 degree shaph outse
;p19: f1 channel - 90 degree shape dulse
;p10: f1 channel duly given by the pulse sequence
;p10: f1 channel duls given by the pulse sequence
;p10: f1 channel duls given by the pulse sequence
;p10: f1 channel duls given by the pulse sequence
;p10: f1 channel duls given by 
    ;pl1 : f1 channel - power level for pulse (default)
                                                                                                                                                                                                     [1 msec]
                                                                                                                                                                                                    [0.3 msec]
                                                                                                                                                                                                    [3 usec]
                                                                                                                                                                                                    [30 msec]
                                                                                                                                                                                                    [4 usec]
   1244 I run Time delay given by the pulse sequence

1255 I run Time delay given by the pulse sequence

1265 I run Time delay given by the pulse sequence

1272 I run Time delay given by the pulse sequence

1283 I run Time delay given by the pulse sequence

1283 I run Time delay given by the pulse sequence
   (33: run time delay given by the pulse sequence
(34: run time delay given by the pulse sequence
(35: run time delay given by the pulse sequence
(37: run time delay given by the pulse sequence
(33: run time delay given by the pulse sequence
```

;cnst2: excitation bandwidth [5ppm] ;cnst3: Tipping angle [-110] ;cnst3: Tippectral width in ½ [=36*sfo3] ;inf1: 1/SW(H) = 2 * DW(H) ;inf2: 1/(2 * SW(H)) = DW(H) ;inf2: 1/(2 * SW(H)) = DW(H) ;l1: maximum TD2 value allowable ;K5: Ben ;K5: Ben ;K1: number of experiments in F1 * 2 ;td2: number of experiments in F1 * 2 ;td2: number of experiments in F1 * 5P ;rpd2: decoupling according to sequence defined by cpdprg2 ;pcpd2: f2 channel (bi_garc_2hl] ;cpd3: f3 channel - 90 degree pulse for decoupling sequence ;cpdprg3: f3 channel - 90 degree pulse for decoupling sequence ;cpdprg3: f3 channel - 90 degree pulse for decoupling sequence ;cpdprg3: f3 channel - 90 degree pulse for decoupling sequence ;cpdprg3: f3 channel bi_garc_2hl2]

;for z-only gradients: ;gpz1: 25% ;gpz2: 35% ;gpz3: 30%

;use gradient files: ;gpnam1: SMSQ10.100 ;gpnam2: SMSQ10.50 ;gpnam3: SMSQ10.100

Appendix S5. Parameter set for 3D C_MN-C_MNH_MH_N SFHMQC-NOESY-HMQC.

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Pulseprogram param	eters of dataset:	D37 [sec]	0	
C:/NMR/sofast/20/p	data/1	D38 [sec]	0	
		DELTA [sec]	0.00002240	
General		DS	16	
PULPROG	sfCNhmqcnoeCNhmqc3d.NCm-NCmHnHm	in0 [sec]	0.00011410	
TD	2048	in10 [sec]	0.00016120	
SWH [Hz, ppm]	13586.96 15.9789	in20 [sec]	0.00011410	
AQ [sec]	0.0753664	in30 [sec]	0.00016120	
RG	128	INF1 [µsec]	228.20	
DW [µsec]	36.800	INF2 [µsec]	228.20	
DE [µsec]	25.00	10	0	
cnst2	5.00000	11	140	
cnst3	90.00000	12	140	
CNST8	3101.8000488	NS	8	
d0 [sec]	0	p4 [µsec]	22.40	
D1 [sec]	0.2000000	TAU [sec]	0.29645202	
D8 [sec]	0.3000001	ZGOPTNS		
d10 [sec]	0	Channel fl		
d11 [sec]	0.03000000	SFO1 [MHz]	850.3039930	
d13 [sec]	0.0000400	O1 [Hz, ppm]	3993.01	4.696
d14 [sec]	0.00400000	NUC1	1H	
d15 [sec]	0.00520000	Pl [µsec]	10.60	
D16 [sec]	0.00020000	p41 [µsec]	1693.52	
D19 [sec]	0.00007400	PLW0 [W, -dBW]	0	1000.00
d20 [sec]	0	PLW1 [W, -dBW]	12	-10.79
D22 [sec]	0	SPNAM 29	Pc9_4_90.pw1693	.5us.cos3230Hz
D23 [sec]	0	SPOAL29	0.500	
D24 [sec]	0	spoffs29 [Hz]	0	
D25 [sec]	0	spw29 [W, -dBW]	0.12035	9.20
D26 [sec]	0	SPNAM 31	Pc9_4_90.pw1693	.5us.cos3230Hz
D27 [sec]	0	SPOAL31	0.500	
D28 [sec]	0	spoffs31 [Hz]	0	
d30 [sec]	0	spw31 [W, -dBW]	0.12035	9.20
D32 [sec]	0	Channel f2		
D33 [sec]	0	SFO2 [MHz]	213.8116328	
D34 [sec]	0	O2 [Hz, ppm]	3527.83	16.500
D35 [sec]	0	NUC2	13C	
D36 [sec]	0	CPDPRG 2	bi_garp_2pl	

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Appendix S5. (continued)

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P3 [µsec]	11.20	
PCPD2 [µsec]	50.00	
PLW2 [W, -dBW]	180	-22.55
PLW12 [W, -dBW]	9.0317	-9.56
PLW30 [W, -dBW]	9.0317	-9.56
PLW31 [W, -dBW]	18.021	-12.56
Channel f3		
SFO3 [MHz]	86.1703179	
O3 [Hz, ppm]	10166.90	118.000
NUC3	15N	
CPDPRG 3	bi_garp_2pl.2	
P21 [µsec]	38.00	
p22 [µsec]	76.00	
PCPD3 [µsec]	240.00	
PLW3 [W, -dBW]	295	-24.70
PLW16 [W, -dBW]	7.3955	-8.69
PLW32 [W, -dBW]	7.3955	-8.69
PLW33 [W, -dBW]	14.756	-11.69
Gradient channel		
GPNAM 1	SMSQ10.100	
GPNAM 2	SMSQ10.50	
GPNAM 3	SMSQ10.100	
GPZ1 [%]	25.00	
GPZ2 [%]	35.00	
GPZ3 [%]	30.00	
P16 [µsec]	1000.00	
p19 [µsec]	500.00	

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Appendix S6. Pulse program for 3D C_{Aro}-C_MH_M SFHMQC-NOESY-HMQC (Topspin ver. 2.1 to 3.5).

go=2 ph31 cpd2:†2 d11 do:f2 mc #0 to 2

F2PH(ip6, id10)

for tonsnin3 0

;for topspin2.0 F1PH(rd10 & rp6 & ip3, id0)

;sthmqcnoesyhmqc3d.Ca-CmHm: 30 HMQC-NUESY-HMQC for Caro(F1)-Cm(F2)Hm(F3) ;H-C(t1)-H -NUE -H-C(t2)-H(t3): C(t1) for aromatic 13C, C(t2) and H(t3) are for methyl :Voulin Xia on 05/05/2016

prosol relations=<triple>

#include <Avance.incl>
#include <Delay.incl>
#include <Grad.incl>

"d11=30m"

"in0 =inf1/2" "in10=inf2/2" "d0=0u" "d10=0u"

"TAU=d8-p3-p16-0.5m-5u"

"d14=1s/(cnst4*2)" ;cnst4=125 "d15=1s/(cnst5*2)" ;cnst5=160

"p41=7.2/(cnst2*bf1/1000000)" /* PC9 pulse length */ "p42=4.875/(cnst2*bf1/1000000)" /* REBURP pulse length */

"spw25=plw1*(pow((p1/p41)/0.125,2))" /* PC9 power level */ "spoff25=bf1*(cnst21/1000000)-o1" /* PC9 offset */

"spw26=plw1*(pow((p1*2/p42)/0.0798,2))" /* REBURP power level */ "spoff26=spoff25" /* REBURP offset */

"spw27=plw1*(pow((p1*(cnst3/90)/p41)/0.125,2))" "spoff27=bf1*(cnst1/1000000)-o1" /* PC9 offset */

"spw28=plw1*(pow((p1*2/p42)/0.0798,2))" "spoff28=spoff27" /* PC9 offset */

"DELTA1=d14-p16-d16-p41*0.5" "DELTA2=p41*0.5-de-4u"

"DELTA3=d15-p16-d16-p41*0.5" "DELTA4=p41*0.5"

aqseq 321

1 d11 ze 2 d11 do:f2 3 d1 pl0:f1 pl2:f2

10u UNBLKGRAD

Su fq=cnst22(bf ppm):f2 ;set 13C to center of 13C freq of aromatci ring [125ppm] (p3 ph1):f2 ph5:gp3 200u

"d20 = d0*2 - p3*0.637*2" "if (d20 < 0) { d20 = 0; }"

"d21 = d10*2 - p3*0.637*2" "if (d21 < 0) { d21 = 0; }"

;HMQC1 (p41:sp25 ph1):f1 p16:gp1 d16

(center (p42:sp26 ph5):f1 (DELTA3 p3 ph3 d20 p3 ph4 DELTA3):f2) p16:gp1

d16

DELTA4 pl1:f1 (p1 ph1):f1 ;(p41:sp25 ph1):f1

;mixing

TAU Su fq=0:f2 ;return back to o2p [16ppm] (p3 ph1):f2 p16:gp3 0.5m p10:f1

;HMQC2

(p41:sp27 ph1):f1 p16:gp1 d16

(center (p42:sp28 ph8):f1 (DELTA1 p3 ph6 d21 p3 ph7 DELTA1):f2)

DELTA2 p16:gp1 d16 pl12:f2 4u BLKGRAD

;FOF tOPSpins.0 ;F1PH(calph(ph3, +90), caldel(d0, +in0)) ;F2PH(calph(ph6, +90), caldel(d10, +in10)) exit ph1 =0 ph2 =1 ph3 =0 2 ph3 =0 2 2 ph4 =0 0 2 2 ph5 =0 0 0 0 1 1 1 1 ph6 =0 0 0 0 0 0 0 0 0 2 2 2 2 2 2 2 2 2 2 ph7 =0 ph8 =0 ph31=0 2 2 0 2 0 0 2 2 0 0 2 0 2 2 0 ;pl1: f1 channel - power level for pulse (default) ;pl2: f2 channel - power level for pulse (default) ;pl2: f2 channel - power level for rhoged 90 degree pulse ;sp25: f1 channel - power level for shaped 90 degree pulse ;sp26: f1 channel - power level for shaped 180 degree pulse ;sp28: f1 channel - power level for shaped 90 degree pulse ;sp28: f1 channel - power level for shaped 180 degree pulse ;spa8: f1 channel - power level for shaped 180 degree pulse ;spa8: f2 channel - power level for shaped 180 degree pulse ;spa8: f2 channel - power level for shaped 180 degree pulse ;spa8: f2 channel - power level for shaped 180 degree pulse ;spa8: f2 channel - power level for shaped 180 degree pulse ;spa8: f2 channel - power level f0 channel ;spnaa2?: Pcy__0y0.1000 ;spnaa2?: Pcy__0y0.1000 ;p1 : f1 channel - 90 degree high power pulse ;p2 : f1 channel - 90 degree high power pulse ;p4 : f2 channel - 90 degree high power pulse ;p4 : f2 channel - 180 degree high power pulse ;p16: homospoil/gradient pulse [1 msec] ;pl: nomospl:/graden/pulse ;p41: f1 channel - 90 degree shaped pulse ;p42: f1 channel - 180 degree shaped pulse ;d0 : incremented delay (F1 in 3D) ;d1 : relaxation delay; 1-5 * T1 [3 usec] ;d2 : 1/((2))XH) ;d8 : mixing time ;d10: incremented delay (F2 in 3D) ;d11: delay for disk I/0 [3 usec] [30 msec] ;d14: 1/(2JCH) [4.0 msec] ;dl4: 1/(2JCH)
;dl6: delay for homospoil/gradient recovery
;d20: run time delay given by the pulse sequence
;d21: run time delay given by the pulse sequence
;cnst1: center of methyl 1H in ppm [0.9] ;cnst2: 1H excitation bandwidth [5] ;cns1: imexcitation bandwidtm [5] ;cns1: floping angle of the 2nd HMQC[110] ;cns1: JOH of methyl [125] ;cns1: IOH of aromatic ring [160] ;cns12: center of aromatic IH chemical shifts [8.5] ;cns122: center of aromatic I3C chemical shifts [125] ;o1p: center of methyl 1H chemical shifts [4.7] ;o2p: center of methyl 13C chemical shifts [16] ;inf1: 1/SW(H) = 2 * DW(H) ;inf2: 1/SW(X) = 2 * DW(X) ;in0: 1/(2 * SW(H)) = DW(H) ;nd0: 2
;n10: 1/(2 * SW(X)) = DW(X) ;nd10: 2 ;NS: 8 * n :DS: 16 ;td1: number of experiments in F1 ;td2: number of experiments in F2 ;FnMODE: States-TPPI (or TPPI) in F1 :FnMODE: States-TPPI (or TPPI) in F2 ;rmuor: States-rpri (U) rpri) in r2 ;cpd2: decoupling according to sequence defined by cpdprg2 ;cpcd2: f2 channel - 90 degree pulse for decoupling sequence ;cpdprg2: f2 channel - bi_garp_2pl

;for z-only gradients: ;gpz1: 25% ;gpz3: 30%

;use gradient files: ;gpnam1: SMSQ10.100 ;gpnam3: SMSQ10.100

Appendix S7. Parameter set for 3D C_{Aro} - C_MH_M SFHMQC-NOESY-HMQC (Bruker Topspin ver. 2.1 to 3.5).

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Pulseprogram par	ameters of datas	et:	Channel f1		
C:/NMR/sofast/10	9/pdata/1		SFO1 [MHz]	850.3039982	
	-		Ol [Hz, ppm]	3998.21	4.702
General			NUC 1	1H	
PULPROG	sfhmqcnoesyhm	lqc3d.am.CCH	p41 [µsec]	2116.90	
TD	2048	-	p42 [µsec]	1433.32	
SWH [Hz, ppm]	13736.26	16.1545	PLW0 [W, -dBW]	0	1000.00
AQ [sec]	0.0745472		PLW1 [W, -dBW]	12	-10.79
RG	90.5		SPNAM 25	Pc9_4_90.1000	
DW [µsec]	36.400		SPOAL25	0.500	
DE [µsec]	25.00		spoffs25 [Hz]	2379.04	
CNST1	1.0000000		spw25 [W, -dBW]	0.036757	14.35
CNST2	4.0000000		SPNAM 26	Reburp.1000	
CNST4	125.0000000		SPOAL26	0.500	
CNST5	160.0000000		<pre>spoffs26 [Hz]</pre>	2379.04	
CNST21	7.5000000		spw26 [W, -dBW]	0.74844	1.26
d0 [sec]	0		SPNAM 27	Pc9_4_90.1000	
D1 [sec]	1.00000000		SPOAL27	0.500	
D8 [sec]	0.3000001		spoffs27 [Hz]	-3147.91	
d10 [sec]	0		spw27 [W, -dBW]	0.036757	14.35
d11 [sec]	0.03000000		SPNAM 28	Reburp.1000	
D16 [sec]	0.00020000		SPOAL28	0.500	
D20 [sec]	0		spoffs28 [Hz]	-3147.91	
D21 [sec]	0.00180000		spw28 [W, -dBW]	0.74844	1.26
DELTA1 [sec]	0.00174155		Channel f2		
DELTA2 [sec]	0.00102945		SFO2 [MHz]	213.8118466	
DELTA3 [sec]	0.00086655		O2 [Hz, ppm]	3741.64	17.500
DELTA4 [sec]	0.00105845		NUC2	13C	
DS	32		CNST22	122.0000000	
in0 [sec]	0.00009350		CPDPRG 2	bi_garp_2pl	
in10 [sec]	0.00011690		P3 [µsec]	11.20	
INF1 [µsec]	187.00		PCPD2 [µsec]	50.00	
INF2 [µsec]	233.80		PLW2 [W, -dBW]	180	-22.55
NS	16		PLW12 [W, -dBW]	9.0317	-9.56
Pl [µsec]	14.50		PLW30 [W, -dBW]	9.0317	-9.56
TAU [sec]	0.29848382		PLW31 [W, -dBW]	36.127	-15.58
dCH [sec]	0.00400000		Gradient channel		
dCH2 [sec]	0.00312500		GPNAM 1	SMSQ10.100	

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GPNAM 3	SMSQ10.100
GPZ1 [%]	11.00
GPZ3 [%]	30.00
P16 [µsec]	1000.00

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Appendix S8. Pulse program for 3D H_NH_{Aro}-C_MH_M SFNOESY-HMQC (Bruker Topspin ver. 2.1 to 3.5).

;stnoesyhmqcid.HnHa-CmHm: NUESY-HMUC for HnHa(F1)-Lm(F2)Hm(F3) ;H(t1)-AUE-H-C(t2)-H(t3): H(t1) for both amide and aromatic 1H, C(t2) and H(t3) are for methyl ;Vulin Xia on 05/69/2016

;note: if setting cnst21 to 1 and cnst22 to 16, the pulse program can also apply for methyl-methyl NOESY

; \$CLASS=HighRes ; \$DIM=3D ; \$TYPE= ; \$SUBTYPE= ; \$COMMENT=

prosol relations=<triple>

#include <Avance.incl>
#include <Delay.incl>
#include <Grad.incl>

"d11=30m"

"in0 =inf1/2" "in10=inf2/2" "d0=3u" "d10=0u" "d14=15/(cnst4*2)"

"TAU=d8-p3-p16-0.5m"

"p41=7.2/(cnst2*bf1/1000000)" /* PC9 pulse length */ "p42=4.875/(cnst2*bf1/1000000)" /* REBURP pulse length */

:cnst4=125

"spw25=plw1*(pow((p1/p41)/0.125,2))" "spoff25=0" /* PC9 offset */

"spw26=plw1*(pow((p1*2/p42)/0.0798,2))" "spoff26=0" /* PC9 offset */

"spw27=plw1*(pow((p1*(cnst3/90)/p41)/0.125,2))" "spoff27=bf1*(cnst1/1000000)-o1" /* PC9 offset */

"spw28=plw1*(pow((p1*2/p42)/0.0798,2))" "spoff28=spoff27" /* PC9 offset */

"DELTA1=d14-p16-d16-p41*0.5" "DELTA2=p41*0.5-de-4u"

aqseq 321

1 d11 ze 2 d11 do:f2 3 d1 pl0:f1 pl3:f3

10u UNBLKGRAD

"d21 = d10*2 - p3*0.637*2" "if (d21 < 0) { d21 = 0; }"

;NOESY p16:gp3 d16

4u fq=cnst21(bf ppm):f1 4u fq=cnst22(bf ppm):f2

4u pl12:f2 5u cpds5:f2 (p41:sp25 ph3):f1 d0 (p22 ph1):f3 d0 (p42:sp26 ph5):f1 3u (p22 ph1):f3 3u

(p41:sp25 ph4):f1 5u do:f2 4u fq=0:f2

;mixing TAU pl2:f2 fq=0:f1 ;0 Hz offset (p3 ph1):f2 pl6:gp3 0.5m

;HMQC2 (p41:sp27 ph1):f1 p16:gp1 d16 (center (p42:sp28 ph8):f1 (DELTA1 p3 ph6 d21 p3 ph7 DELTA1):f2) DELTA2 p16:gp1 d16 pl12:f2 4u BLKGRAD qo=2 ph31 cpd2:f2 d11 do:f2 mc #0 to 2 ;for topspin2.0 F1PH(rd10 & rp6 & ip3, id0) F2PH(ip6, id10) :for topspin3.0 ;F1PH(calph(ph3, +90), caldel(d0, +in0)) ;F2PH(calph(ph6, +90), caldel(d10, +in10)) exit ph1 =0 ph2 =1 ph3 =0 2 ph4 =0 ph5 =0 0 0 0 0 1 1 1 1 ph6 =0 0 2 2 ph7 =0 ph8 =0 ph31=0 2 2 0 2 0 0 2 ;pl1 : f1 channel - power level for pulse (default) ;pl1 : f1 channel - power level for pulse (default) ;pl12 : f2 channel - power level for pulse (default) ;pl12 : f2 channel - power level for CPD/B8 decoupling ;sp25: f1 channel - power level for shaped 90 degree pulse ;sp26: f1 channel - power level for shaped 90 degree pulse ;sp27: f1 channel - power level for shaped 90 degree pulse ;sp28: f1 channel - power level for shaped 180 degree pulse ;sp28: f1 channel - power level for shaped 180 degree pulse ;spanz25: Redurp.1000 ;spnaz27: Rod_4.90.1000 ;spnaz27: Rod_4.90.1000 :spnam28: Beburp.1000
;pl :fl channel - 90 degree high power pulse
;p2 :fl channel - 180 degree high power pulse
;p3 :fl channel - 180 degree high power pulse
;p4 :f2 channel - 180 degree high power pulse
;p6 :fonsopol/gradient pulse [1 msec] ;p41: f1 channel - 90 degree shaped pulse ;p41: f1 channel - 180 degree shaped pulse ;p42: f1 channel - 180 degree shaped pulse ;d0 : incremented delay (F1 in 30) ;d1 : relaxation delay; 1-5 * T1 [3 usec] :d2 : 1/((2J)XH) ;02 : 1/((2)/AN)
;d8 : mixing time
;d10: incremented delay (F2 in 30)
;d11: delay for disk I/0
;d14: 1/(2)CH) [3 usec] [30 msec] [3.7 msec] ;dl: 1/(22H)
;dl: delay for homospoil/gradient recovery
;dl: run time delay given by the pulse sequence
;cnst1: exter of methyl lH in ppm [0.9]
;cnst2: H excitation bandwidth [5]
;cnst3: flipping angle of the 2nd HMQC[110]
;cnst5: 10d of aromatic ring [160]
;cnst21: center of aromatic 1H chemical shifts [8.5]
;cnst22: center of aromatic 1A chemical shifts [125]
;cp1: center of methyl lA chemical shifts [11]
;cp2: center of methyl l3C chemical shifts [16] ;inf1: 1/SW(H) = 2 * DW(H) ;inf2: 1/SW(X) = 2 * DW(X) ;in0: 1/(2 * SW(H)) = DW(H) :nd0: 2 ;nl0: 2 ;nl0: 1/(2 * SW(X)) = DW(X) ;nd10: 2 ;NS: 4 * n ;DS: 8 ;td1: number of experiments in F1 ;tdl: number of experiments in F1 ;td2: number of experiments in F2 ;FnMODE: States-TPPI (or TPPI) in F1 ;FnMODE: States-TPPI (or TPPI) in F2 ;cpd2: decoupling according to sequence defined by cpdprg2 ;pcpd2: f2 channel - 90 degree pulse for decoupling sequence ;cpdprg2: f2 channel - bi_garp_128

;for z-only gradients: ;gpz1: 25% ;gpz3: 30%

;use gradient files: ;gpnam1: SMSQ10.100 ;gpnam3: SMSQ10.100

Appendix S9. Parameter set for 3D H_NH_{Aro}-C_MH_M SFNOESY-HMQC.

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C:/NNR/sofast/308/pdata/1 P41 [usec] 1410.59 P42 [usec] 1433.32 P42 [usec] 1433.32 PULPROG sfnoesyhmgc3d.Am.HCH SPNIM 25 Pcf_4_0.1000 TD 1356.06 15.9789 spoffs25 [H2] 0 AQ [sec] 0.0499008 spoffs25 [H2] 0 14.43 RG 90.5 spoffs25 [H2] 0 14.43 RG 90.5 spoffs25 [H2] 0 14.43 RG 90.5 spoffs26 [H2] 0 1.13 DE [usec] 25.00 spoffs26 [H2] 0 1.13 CNST1 1.000000 spoffs27 [H2] 0.77141 1.13 CNST2 90.000000 SPOAL26 0.500 14.43 D1 [sec] 0.300000 spoffs27 [H2] -3147.91 1.13 CNST3 90.000000 spoffs27 [H2] -3147.91 1.13 D1 [sec] 0.3000001 spoffs28 [H2] -3147.91 1.13 D1 [sec] 0.3000001 spoffs28 [H2] -3147.91 1.13 D1 [sec] 0.0010000 spoffs28 [H2] -3147.91 1.13 D1 [sec] 0.001000 spoffs28 [H2] -3147.91 1.13 D1 [sec]	Pulseprogram param	eters of dataset	:	p41 [ucod]	2116 00	
General PKV [jakc] 1435.3 PULPROC sfnoesyhmqc3d.Am.HCH PKN [W, ~dBM] 12 -10.79 TD 1356 SPANA 25 PS_4_90.1000 -10.79 TD 1356 SPANA 25 PS_4_90.1000 -10.79 SWH [Jz, ppm] 13586.96 15.9789 spoffa25 [Hz] 0 -10.8000 RG 90.5 SPANA 26 Reburp.1000 -10.79 DW [jasec] 36.800 SPANA 26 Reburp.1000 -10.8000 DE [jasec] 36.800 spoffa26 [Hz] 0 -10.79 CNST1 1.000000 spoffa27 [Mz] 0.7141 1.13 CNST2 4.000000 SPANA 27 PC9_4_90.1000 -500 CNST4 125.000000 SPANA 27 PC9_4_90.1000 -500 CNST3 0.0000300 SPANA 28 Reburp.1000 -500 Spartal 2.000000 SPANA 28 Reburp.1000 -500 Spartal 0.001000 SPANA 28 Reburp.1000 -500	C:/NMR/sofast/308/	pdata/1		p41 [µsec]	1422 22	
General FINI [N, -dBW] 12 -10.79 PULPROG sfnesyhmqc3d.Am.HCH SPNAM 25 PC_4_90.1000 TD 1356 SPNAM 25 0.500 SWH [Hz, ppm] 1356.15.9789 spOffa25 [Hz] 0 AQ [sec] 0.4099008 spW25 [N, -dBW] 0.036033 14.43 RG 90.5 SPNA 26 Reburp.1000 14.43 RG 90.5 SPNA 26 Reburp.1000 14.43 RG 90.5 SPNA 26 Reburp.1000 14.43 RG 90.500000 SPNA 26 Reburp.1000 113.0 CNST1 1.000000 SPNA 27 PC_4_90.1000 14.43 CNST2 90.000000 SPNA 27 0.500 14.43 D1 [sec] 0.3000000 SPNA 28 Reburp.1000 14.43 D1 [sec] 0.30000001 SPNA 28 Reburp.1000 14.43 D1 [sec] 0.3000000 SPNA 28 Reburp.1000 14.43 D1 [sec] 0.3000000 SPO128 N27<				P42 [µsec] DIWO [W _dBW]	1455.52	1000 00
PULPROG shoesyhmqc3d.Am.HCH SPNAM 25 Pc9_4_90.100 TD 1356 SPNAM 25 Pc9_4_90.100 TD 1356.96 15.9789 spoffs25 [Hz] 0 SNH [Hz, ppm] 13586.96 15.9789 spoffs25 [Hz] 0 AQ [sec] 0.0499008 spoffs26 [Hz] 0 0.036033 14.43 RG 90.5 SPNAM 26 Reburp.1000 DW [µsec] 36.800 spoffs26 [Hz] 0 DE [µsec] 25.00 spoffs27 [Hz] 0.77141 1.13 CNST1 1.000000 SPNAM 27 Pc9_4_90.1000 CNST4 CNST2 4.000000 SPOAL27 0.500 CNST4 D1 [sec] 0.2000300 spoffs27 [Hz] -3147.91 4.43 D1 [sec] 0.3000001 SPOAL28 0.500 CNST4 D1 [sec] 0.3000000 Channel f2 11.3 1.443 D1 [sec] 0.0012000 Channel f2 13.8118466 17.500 D21 [sec] 0.001204155 NCC	General			PIWU [W, -dBW]	12	-10 79
TD 1356 SPOLZS 0.500 SWH Hz, ppm] 1356.96 15.9789 spoff225 [12] 0 AQ [sec] 0.0499008 spoff225 [12] 0 AQ [sec] 0.0590 spxAz 5 (W, -dBW] 0.036033 14.43 RG 90.5 SPOAL26 0.036033 14.43 DW [µsec] 36.800 SPOAL26 0.036033 14.43 DW [µsec] 36.800 spoffs27 [12] 0.500 I.13 CNST1 1.0000000 spoffs27 [12] 0.7114 1.13 CNST2 4.0000000 spoffs27 [12] -3147.91 I.500 CNST4 9.0000000 spoffs27 [12] -3147.91 I.50 d0 [sec] 0.20000000 spoffs28 [12] -3147.91 I.50 B8 [sec] 0.0002000 SpoIL28 SpoIL28 I.500 D1 [sec] 0.00174155 SpoIL28 I.21.9114 I.13 D1 [sec] 0.0017015 SpoIL28 I.22.000000 I.22.000000 D1 [sec] <	PULPROG	sfnoesyhmqc3d.A	m.HCH	SDNAM 25	Pc9 4 90 1000	-10.75
SWH [Hz, ppm] 13586.96 15.9789 spoffs25 [Hz] 0 AQ [sec] 0.0499008 spw25 [W, -dBW] 0.36033 14.43 RG 90.5 SPNA 26 Reburp.1000 DW [isec] 36.800 SPNA 26 Reburp.1000 DE [isec] 25.00 SpOL26 0.500 CNST1 1.000000 spW26 [W, -dBW] 0.7141 1.13 CNST2 4.000000 SPOAL27 0.500 CNST3 90.000000 SPOAL27 0.500 CNST4 125.0000000 SPOAL28 0.500 D1 [sec] 0.2000000 SPOAL27 0.500 D1 [sec] 0.3000000 SPOAL28 Reburp.1000 D8 [sec] 0.3000000 SPOAL28 0.510 D1 [sec] 0.0014000 Channel f2 D1 [sec] 0.0012000 SPO2 [ME2] 31.64 17.500 DELTA1 [sec] 0.001245 <t< td=""><td>TD</td><td>1356</td><td></td><td>SPOAL25</td><td>0.500</td><td></td></t<>	TD	1356		SPOAL25	0.500	
AQ [sec] 0.0499008 spw25 [W, -dBH] 0.036033 14.43 RG 90.5 SPNA 26 Reburp.1000 DW [µsec] 36.800 SPOAL26 0.500 DE [µsec] 25.00 spoffs26 [Hz] 0 CNST1 1.000000 spw26 [W, -dBW] 0.77141 1.13 CNST2 4.000000 SPNA 27 Peg_4_90.1000 CNST3 90.000000 SPOAL27 0.500 - CNST4 125.000000 SPNA 27 Peg_4_90.1000 - CNST4 125.000000 SPNA 28 Reburp.1000 - CNST4 125.000000 SPNA 28 Reburp.1000 - CNST4 0.300000 SPNA 28 Reburp.1000 - Bs(sc] 0.0300000 SPMA 28 Reburp.1000 - Ch1 [sec] 0.0300000 SPM28 [Hz] -3147.91 - Ch1 [sec] 0.0040000 SPM28 [Hz] -3147.91 - Ch2 [sec] 0.0018000 Channel f2 - - - D16 [sec] 0.00174155 NUC2 13C	SWH [Hz, ppm]	13586.96	15.9789	spoffs25 [Hz]	0	
RG 90.5 SPNAN 26 Reburp.1000 DW [µsec] 36.800 SPOAL26 0.500 DE [µsec] 25.00 spoffs26 [Hz] 0. CNST1 1.000000 SpoAL27 0.57141 1.13 CNST2 4.000000 SPOAL27 0.500 - CNST3 90.0000000 SpOAL27 0.500 - CNST4 125.0000000 spoffs27 [Hz] -3147.91 - d0 [sec] 0.0000000 SPNAN 28 Reburp.1000 - D1 [sec] 0.3000000 SPNAN 28 Reburp.1000 - d1 [sec] 0.3000000 SPNAN 28 Reburp.1000 - d1 [sec] 0.3000000 SPNAN 28 Reburp.1000 - d1 [sec] 0.0300000 SPNAN 28 Reburp.1000 - D1 [sec] 0.00120000 SPNAN 28 Reburp.1000 - D21 [sec] 0.0012000 SPS2 [Hz] -3141.91 -1.13 D11 [sec] 0.0012015 Rus SPOEL27 122.000001 D12 [sec] 0.00120245 CNST21	AQ [sec]	0.0499008		spw25 [W, -dBW]	0.036033	14.43
DW [µsec] 36.800 STOAL26 0.500 DE [µsec] 25.00 spoffs26 [Hz] 0 CNST1 1.000000 spv26 [W, -dBW] 0.77141 1.13 CNST2 4.000000 spv26 [W, -dBW] 0.77141 1.13 CNST3 90.000000 SPAM 27 Pc 9_40.1000 CNST4 CNST4 125.000000 Spoffs27 [Hz] -3147.91 -3147.91 d0 [sec] 0.3000001 Spoffs28 [Hz] -3147.91 -3147.91 D1 [sec] 0.3000001 Spoffs28 [Hz] -3147.91 -3147.91 d11 [sec] 0.0040000 Spoffs28 [Hz] -3147.91 -3147.91 d14 [sec] 0.0040000 Spoffs28 [Hz] -3147.91 -3147.91 d14 [sec] 0.0040000 SPO2 [Mz] 213.818466 -22.000000 D21 [sec] 0.0018000 C2 [Hz, ppm] 3741.64 17.500 D21 [sec] 0.0012945 CNST22 122.000000 - D3 [sec] 0.0001450 PC PD2 [secc] 50.00 -	RG	90.5		SPNAM 26	Reburp.1000	
DE [µsec] 25.00 spoffs26 [Hz] 0 CNST1 1.000000 spv26 [W, -dBW] 0.7141 1.13 CNST2 4.000000 SPNAH 27 Pc9_490.1000 CNST3 90.0000000 spoffs27 [Hz] -3147.91 CNST4 125.000000 spv27 [W, -dBW] 0.36033 14.43 D1 [sec] 0.20000001 SPNAH 28 Reburp.1000 14.43 D1 [sec] 0.3000001 Spoffs28 [Hz] -3147.91 .500 d10 [sec] 0.3000001 Spoffs28 [Hz] -3147.91 .500 d11 [sec] 0.3000000 spoffs28 [Hz] -3147.91 .113 d14 [sec] 0.3000000 spoffs28 [Hz] -3147.91 .500 d11 [sec] 0.0040000 Channel f2 .500 .500 D15 [sec] 0.00174155 NUC2 13C .500 D21 [sec] 0.0012945 NUC2 122.000000 .500 Spoffs2 [µsec] 0.0011690 PCPD2 [µsec] 11.20 INF2 [µsec] 0.00011690 PCPD2 [µsec] 50.00 INF2 [µsec] 0.0011690 PCPD2 [µsec] 50.00 INF2 [µsec] 0.0011690 PCPD2 [µsec] 50.00 INF2 [µsec] 23.80	DW [µsec]	36.800		SPOAL26	0.500	
CNST1 1.000000 spw26 [W, -dW] 0.77141 1.13 CNST2 4.000000 SPNM 27 Pc9_4_90.1000 CNST3 90.000000 SPOAL27 0.500 CNST4 125.0000000 spoffs27 [Hz] -3147.91 d0 [sec] 0.0000300 spv27 [W, -dW] 0.36033 14.43 D1 [sec] 0.2000000 SPOAL28 0.500	DE [µsec]	25.00		spoffs26 [Hz]	0	
CNST2 4.000000 SPNAM 27 Pc9_4_90.1000 CNST3 90.000000 SPOAL27 0.500 CNST4 125.000000 spoffs27 [Hz] -3147.91 d0 [sec] 0.0000300 spv27 [W, -dBW] 0.036033 14.43 D1 [sec] 0.2000000 SPNAM 28 Reburp.1000 D8 [sec] 0.3000001 SPOAL28 0.500 d10 [sec] 0 spoffs28 [Hz] -3147.91 d11 [sec] 0.0300000 SPV28 [W, -dBW] 0.77141 1.13 d14 [sec] 0.0040000 SP02 [Hz] 213.8118466 17.500 D16 [sec] 0.0012000 SPO2 [Hz] 213.8118466 17.500 D21 [sec] 0.0012945 CNST2 122.00000 IS D21 [sec] 0.0013070 CPDPRG 2 bi_garp_2p1 IS in10 [sec] 0.0011690 P3 [µsec] 11.20 INT NS 8 PLW12 [W, -dBW] 9.017 -9.56 NS 8 PLW12 [W, -dBW] 9.017 -9.56 NS 8 PLW12 [W, -dBW] 9.0177 -9.5	CNST1	1.000000		spw26 [W, -dBW]	0.77141	1.13
CNST3 90.000000 SPOAL27 0.500 CNST4 125.000000 spoffs27 [Hz] -3147.91 d0 [sec] 0.0000300 spw27 [W, -dBW] 0.36033 14.43 D1 [sec] 0.2000000 SPVA 28 Reburp.1000 14.43 D8 [sec] 0.3000001 SPOAL28 0.500 14.43 d10 [sec] 0.3000000 Spoffs28 [Hz] -3147.91 .13 d11 [sec] 0.3000000 Spoffs28 [Hz] -3147.91 .13 d14 [sec] 0.0300000 Spoffs28 [Hz] -3147.91 .13 d14 [sec] 0.0300000 Spoffs28 [Hz] -3147.91 .13 d14 [sec] 0.000000 Spoffs28 [Hz] -3147.91 .13 D16 [sec] 0.0002000 Spoffs28 [Hz] -7141 .13 D21 [sec] 0.00174155 NUC2 13c	CNST2	4.000000		SPNAM 27	Pc9 4 90.1000	
CNST4 125.000000 spoffs27 [Hz] -3147.91 d0 [sec] 0.0000300 spw27 [W, -dBW] 0.036033 14.43 D1 [sec] 0.2000000 SPNAM 28 Reburp.1000 Image: 2000000 SPNAM 28 Reburp.1000 Image: 200000 Spoffs28 [Hz] -3147.91 Image: 200000 Spoffs28 [Hz] Spoffs28 [Hz]	CNST3	90.0000000		SPOAL27	0.500	
d0 [sec]0.0000300spw27 [W, -dBW]0.03603314.43D1 [sec]0.2000000SPNA 28Reburp.1000D8 [sec]0.3000001SPOAL280.500d10 [sec]0Spoff28 [H2]-3147.91-113d11 [sec]0.0300000spw28 [W, -dBW]0.71411.13d14 [sec]0.00400000Cannel f21.131.13D16 [sec]0.0012000SF02 [MH2]213.81184667.500D21 [sec]0.00174155NUC213C	CNST4	125.0000000		spoffs27 [Hz]	-3147.91	
D1 [sec]0.200000SPNAM 28Reburp.1000D8 [sec]0.3000001SPOAL280.500d10 [sec]0spoffs28 [Hz]-3147.91d11 [sec]0.0300000spw28 [W, -dBW]0.771411.13d14 [sec]0.00400000Channel f2D16 [sec]0.00120000SPO2 [MHz]3741.6417.500D21 [sec]0.01074155NUC213CDELTA1 [sec]0.0102945CNST22122.000000DS8CPDPRG 2si_garp_2p1in0 [sec]0.0011690PDPRG 5garpIn16 [sec]0.0011690PDPRG 511.20INF1 [µsec]23.80PUW2 [W, -dBW]9.0317-9.56NS8PUW2 [W, -dBW]9.0317-9.56P1 [µsec]14.50PUW3 [W, -dBW]9.0317-9.56TAU [sec]0.2984883PUW3 [W, -dBW]9.0317-9.56TAU [sec]0.2984883PUW3 [W, -dBW]6.1703179SF01 [MHz]S50.3039824.702SF03 [MHz]6.1703179O1 [Hz, ppm]398.214.702Q1 [Hz, pm]10.66.90118.000NUC1HNUC3S50S50S50S50NUC1HS60004P22 [µsec]64.00S50	d0 [sec]	0.0000300		spw27 [W, -dBW]	0.036033	14.43
D8 [sec]0.3000001SPOAL280.500d10 [sec]0.300000spoffs28 [H2]-3147.91d11 [sec]0.300000Spo8 [W, -dBW]0.771411.13d14 [sec]0.0040000Chanel f213.811846617.500D16 [sec]0.0012000SF02 [M2]3741.6417.500D21 [sec]0.00174155NUC2122.00000017.500DELTA1 [sec]0.0012945CNST22122.00000012.000000D58CPDPRG 2bi_gar_2F11.50in0 [sec]0.0013070CPDPRG 2garp1.20in10 [sec]0.0011690PSD2 [µsec]11.201.21INF1 [µsec]21.40PCD2 [µsec]50.001.255NS8PLW12 [W, -dBW]9.0317-9.56P1 [µsec]14.50PLW31 [W, -dBW]9.0317-9.56TAU [sec]0.2984883PLW31 [W, -dBW]9.0317-9.56TAU [sec]850.303982Sr03 [MH2]86.1703179SF01 [MH2]398.214.702Sf03 [M2]81.703179O1 [f2, ppm]398.214.70203 [f2, ppm]1016.90118.000NUC1HSe00004NUC35555SF03 [MH2]8.6000044.702Si55SF03 [MH2]398.214.702Si [µsec]64.00	D1 [sec]	0.2000000		SPNAM 28	Reburp.1000	
d10 [sec] 0 spoffs28 [Hz] -3147.91 d11 [sec] 0.0300000 spw28 [W, -dBW] 0.7141 1.13 d14 [sec] 0.0040000 Chanel f2	D8 [sec]	0.3000001		SPOAL28	0.500	
d11 [sec] 0.030000 spv28 [W, -dBW] 0.77141 1.13 d14 [sec] 0.0040000 Chanel f2 1 1 D16 [sec] 0.002000 SF02 [MHz] 213.8118466 17.500 D21 [sec] 0.0018000 O2 [Hz, ppm] 3741.64 17.500 DELTA1 [sec] 0.0174155 NUC2 13C 1 DELTA2 [sec] 0.00102945 CNST22 122.000000 1 DS 8 CPDPRG 2 bi_garp_2p1 - in0 [sec] 0.0011690 CPDPRG 5 garp - in10 [sec] 0.0011690 PCPD2 [gsec] 11.20 - INF1 [µsec] 23.80 PLW2 [W, -dBW] 180 -22.55 NS 8 PLW2 [W, -dBW] 9.0317 -9.56 P1 [µsec] 14.50 PLW3 [W, -dBW] 9.0317 -9.56 TAU [sec] 0.2948883 PLW3 [W, -dBW] 9.0317 -9.56 TAU [sec] 850.303982 SF03 [MHz] 36.127 -15.58 Channel f3 SF03 [MHz] 9.61703179 118.000	d10 [sec]	0		spoffs28 [Hz]	-3147.91	
d14 [sec] 0.0040000 Channel f2 D16 [sec] 0.002000 SF02 [ME] 21.818466 D21 [sec] 0.018000 O2 [Hz, ppm] 3741.64 17.500 DELTA1 [sec] 0.0174155 NUC2 13C 16 DELTA2 [sec] 0.0102945 SNS72 122.000000 16 DS 8 CPDPRG 2 $3rgp_2$ 17.500 in0 [sec] 0.0011690 CPDPRG 5 garp 17.500 INF1 [µsec] 0.0011690 P3 [µsec] 11.20 17.500 INF2 [µsec] 23.80 P102 [µsec] 50.00 -22.55 NS 8 P102 [µsec] 9.0317 -9.56 TAU [sec] 0.2948883 P100 [W, -dBW] 9.0317 -9.56 TAU [sec] 0.2948883 P100 [W, -dBW] 9.0317 -9.56 Channel f1 SF03 [MHz] 86.1703179 15.58 G1 [Hz, ppm] 398.21 4.702 $03 [Hz, ppm]$ 10166.90 18.000 NuC1 H 6.00004 50.0004 50.0004 50.000	d11 [sec]	0.03000000		spw28 [W, -dBW]	0.77141	1.13
D16 [sec] 0.002000 SF02 [ME2] 213.8118466 D21 [sec] 0.018000 02 [Hz, ppm] 3741.64 17.500 DELTA1 [sec] 0.00174155 NUC2 13C Intermediate DELTA2 [sec] 0.00102945 CNST22 122.000000 Intermediate DS 8 CPDPRG 2 isgarp_2pl Intermediate in0 [sec] 0.0011690 CPDPRG 5 garp Intermediate INF1 [µsec] 0.0011690 PGPD2 [µsec] 11.20 Intermediate INF2 [µsec] 23.80 PU2 [µsec] 50.00 Intermediate NS 8 PU2 [µsec] 9.0317 -9.56 P1 [µsec] 14.50 PU30 [Ŵ, -dBW] 9.0317 -9.56 TAU [sec] 0.2984883 PU31 [Ŵ, -dBW] 9.0317 -9.56 Channel f1 Funder f3 Funder f3 Funder f3 Funder f3 SF01 [MH2] 899.21 4.702 03 [Hz, ppm] 61.60.90 118.000 NUC1 IH NUC3 15N Funder f3 Funder f3 Funder f3 Funder f3 Funder f3 <td>d14 [sec]</td> <td>0.00400000</td> <td></td> <td>Channel f2</td> <td></td> <td></td>	d14 [sec]	0.00400000		Channel f2		
D21 [sec] 0.0018000 02 [Hz, ppm] 3741.64 17.500 DELTA1 [sec] 0.00174155 NUC2 13C Immediate DELTA2 [sec] 0.00102945 CNST22 122.000000 Immediate DS 8 CPDPRG 2 bi_garp_2pl Immediate in0 [sec] 0.0011690 CPDPRG 5 garp Immediate INF1 [µsec] 0.0011690 PCPD2 [µsec] 50.00 Immediate INF2 [µsec] 23.80 PLW2 [W, -dBW] 180 -22.55 NS 8 PLW2 [W, -dBW] 9.0317 -9.56 TAU [sec] 14.50 PLW3 [W, -dBW] 9.0317 -9.56 TAU [sec] 0.2984883 PLW3 [W, -dBW] 9.0317 -9.56 TAU [sec] 0.2984883 PLW3 [W, -dBW] 36.127 -15.58 Channel f1 F F F F 18.000 NUC1 3998.21 4.702 O3 [Hz, ppm] 86.1703179 NUC1 IH NUC3 SN F F NUC1 8.600004 F22 [µsec] 64.00 <	D16 [sec]	0.00020000		SFO2 [MHz]	213.8118466	
DELTA1 [sec] 0.00174155 NUC2 13C DELTA2 [sec] 0.0102945 CNST22 122.000000 DS 8 CPDPRG 2 b_garp_2pl in0 [sec] 0.0013070 CPDPRG 5 garp in10 [sec] 0.0011690 P3 [µsec] 11.20 INF1 [µsec] 261.40 PCPD2 [µsec] 50.00 INF2 [µsec] 23.80 PLW2 [W, -dBW] 80 -22.55 NS 8 PLW2 [W, -dBW] 9.0317 -9.56 P1 [µsec] 14.50 PLW3 [W, -dBW] 9.0317 -9.56 TAU [sec] 0.2984883 PLW3 [W, -dBW] 9.0317 -9.56 Channel f1 E FCT FCT -15.58 SF01 [MH2] 850.3039982 SF03 [MH2] 86.1703179 -15.58 O1 [Hz, ppm] 398.21 4.702 SF03 [MH2] 86.1703179 NUC1 IH NUC3 15N -15.80 NUC1 8.600004 P22 [µsec] 64.00 -18.00	D21 [sec]	0.00180000		O2 [Hz, ppm]	3741.64	17.500
DELTA2 [sec] 0.00102945 CNST22 122.000000 DS 8 CPDPRG 2 bi_garp_2pl in0 [sec] 0.0013070 CPDPRG 5 garp in10 [sec] 0.0011690 P3 [µsec] 11.20 INF1 [µsec] 261.40 PCPD2 [µsec] 50.00 INF2 [µsec] 233.80 PLW2 [W, -dBW] 80 -22.55 NS 8 PLW2 [W, -dBW] 9.0317 -9.56 P1 [µsec] 14.50 PLW3 [W, -dBW] 9.0317 -9.56 TAU [sec] 0.2984883 PLW3 [W, -dBW] 9.0317 -9.56 Channel f1 E FUS3 [W, -dBW] 36.127 -15.58 SF01 [MH2] 850.3039982 SF03 [MH2] 86.1703179 -15.58 O1 [Hz, ppm] 398.21 4.702 O3 [Hz, ppm] 10166.90 118.000 NUC1 1H NUC3 15N - - NUC1 8.600004 P22 [µsec] 64.00 -	DELTA1 [sec]	0.00174155		NUC2	13C	
DS 8 CPDPRG 2 bi_garp_2pl in0 [sec] 0.0013070 CPDPRG 5 garp in10 [sec] 0.0011690 P3 [µsec] 11.20 INF1 [µsec] 261.40 PCPD2 [µsec] 50.00 INF2 [µsec] 233.80 PLW2 [W, -dBW] 80.0 -22.55 NS 8 PLW12 [W, -dBW] 9.0317 -9.56 TAU [sec] 0.2984883 PLW30 [W, -dBW] 9.0317 -9.56 Channel f1 PLW31 [W, -dBW] 36.127 -15.58 SF01 [MH2] 850.303982 SF03 [MH2] 86.1703179 O1 [Hz, ppm] 398.21 4.702 O3 [Hz, ppm] 10166.90 118.000 NUC1 14 NUC3 15N P2 [µsec] 54.00	DELTA2 [sec]	0.00102945		CNST22	122.0000000	
in0 [sec] 0.0013070 CPDPRG 5 garp in10 [sec] 0.0011690 P3 [µsec] 11.20 INF1 [µsec] 261.40 PCPD2 [µsec] 50.00 INF2 [µsec] 233.80 PLW2 [W, -dBW] 80 -22.55 NS 8 PLW12 [W, -dBW] 9.0317 -9.56 TAU [sec] 0.2984883 PLW30 [W, -dBW] 9.0317 -9.56 Channel f1 Channel f3 -15.58 SF01 [MHz] 850.303982 SF03 [MHz] 86.1703179 O1 [Hz, ppm] 399.21 4.702 O3 [Hz, ppm] 10166.90 118.000 NUC1 1H NUC3 15N -15.58 CNST21 8.600004 P22 [µsec] 64.00	DS	8		CPDPRG 2	bi_garp_2pl	
in10 [sec] 0.00011690 P3 [µsec] 11.20 INF1 [µsec] 261.40 PCPD2 [µsec] 50.00 INF2 [µsec] 233.80 PLW2 [W, -dBW] 180 -22.55 NS 8 PLW12 [W, -dBW] 9.0317 -9.56 P1 [µsec] 14.50 PLW30 [W, -dBW] 9.0317 -9.56 TAU [sec] 0.29848833 PLW31 [W, -dBW] 36.127 -15.58 Channel f1 Channel f3 -15.58 -15.58 SF01 [MH2] 850.3039982 SF03 [MH2] 86.1703179 O1 [Hz, ppm] 3998.21 4.702 O3 [Hz, ppm] 10166.90 118.000 NUC1 1H NUC3 15N -15.58 CNST21 8.600004 P22 [µsec] 64.00	in0 [sec]	0.00013070		CPDPRG 5	garp	
INF1 [μsec] 261.40 PCP2 [μsec] 50.00 INF2 [μsec] 233.80 PLW2 [W, -dBW] 180 -22.55 NS 8 PLW12 [W, -dBW] 9.0317 -9.56 P1 [μsec] 14.50 PLW30 [W, -dBW] 9.0317 -9.56 TAU [sec] 0.29848833 PLW31 [W, -dBW] 36.127 -15.58 Channel f1 Channel f3 -15.58 -15.58 SF01 [MH2] 850.3039982 SF03 [MH2] 86.1703179 O1 [Hz, ppm] 3998.21 4.702 O3 [Hz, ppm] 10166.90 118.000 NUC1 1H NUC3 15N	in10 [sec]	0.00011690		P3 [µsec]	11.20	
INF2 [µsec] 233.80 PLW2 [W, -dBW] 180 -22.55 NS 8 PLW12 [W, -dBW] 9.0317 -9.56 P1 [µsec] 14.50 PLW30 [W, -dBW] 9.0317 -9.56 TAU [sec] 0.29848833 PLW31 [W, -dBW] 36.127 -15.58 Channel f1 Channel f3 - - SF01 [MH2] 850.3039982 SF03 [MH2] 86.1703179 O1 [Hz, ppm] 3998.21 4.702 O3 [Hz, ppm] 10166.90 118.000 NUC1 1H NUC3 15N - CNST21 8.600004 P22 [µsec] 64.00	INF1 [µsec]	261.40		PCPD2 [µsec]	50.00	
NS 8 PLW12 [W, -dBW] 9.0317 -9.56 P1 [μsec] 14.50 PLW30 [W, -dBW] 9.0317 -9.56 TAU [sec] 0.29848833 PLW31 [W, -dBW] 36.127 -15.58 Channel f1 Channel f3 -9.56 -9.56 SF01 [MHz] 850.3039982 SF03 [MHz] 86.1703179 01 [Hz, ppm] 3998.21 4.702 O3 [Hz, ppm] 10166.90 118.000 NUC1 1H NUC3 15N	INF2 [µsec]	233.80		PLW2 [W, -dBW]	180	-22.55
P1 [μsec] 14.50 PLW30 [W, -dBM] 9.0317 -9.56 TAU [sec] 0.29848833 PLW31 [W, -dBM] 36.127 -15.58 Channel f1 Channel f3 Channel f3 - SF01 [MHz] 850.3039982 SF03 [MHz] 86.1703179 01 [Hz, ppm] 3998.21 4.702 03 [Hz, ppm] 10166.90 118.000 NUC1 1H NUC3 15N - CNST21 8.600004 P22 [μsec] 64.00	NS	8		PLW12 [W, -dBW]	9.0317	-9.56
TAU [sec] 0.29848883 PLW31 [W, -dBW] 36.127 -15.58 Channel f1 Channel f3 Channel f3 Channel f3 SF01 [MHz] 850.3039982 SF03 [MHz] 86.1703179 01 [Hz, ppm] 3998.21 4.702 03 [Hz, ppm] 10166.90 118.000 NUC1 1H NUC3 15N CNST21 8.600004 P22 [µsec] 64.00	Pl [µsec]	14.50		PLW30 [W, -dBW]	9.0317	-9.56
Channel f1 Channel f3 SF01 [MHz] 850.3039982 SF03 [MHz] 86.1703179 O1 [Hz, ppm] 3998.21 4.702 O3 [Hz, ppm] 10166.90 118.000 NUC1 1H NUC3 15N CNST21 8.600004 P22 [µsec] 64.00	TAU [sec]	0.29848883		PLW31 [W, -dBW]	36.127	-15.58
SF01 [MHz] 850.3039982 SF03 [MHz] 86.1703179 01 [Hz, ppm] 3998.21 4.702 03 [Hz, ppm] 10166.90 118.000 NUC1 1H NUC3 15N CNST21 8.600004 P22 [µsec] 64.00	Channel fl			Channel f3		
O1 [Hz, ppm] 3998.21 4.702 O3 [Hz, ppm] 10166.90 118.000 NUC1 1H NUC3 15N CNST21 8.600004 P22 [µsec] 64.00	SFO1 [MHz]	850.3039982		SFO3 [MHz]	86.1703179	
NUC1 1H NUC3 15N CNST21 8.600004 P22 [µsec] 64.00	O1 [Hz, ppm]	3998.21	4.702	O3 [Hz, ppm]	10166.90	118.000
CNST21 8.6000004 P22 [µsec] 64.00	NUC1	1H		NUC3	15N	
	CNST21	8.6000004		P22 [µsec]	64.00	

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Wed Jul 20 15:34:13 CDT 2016

PLW3 [W, -dBW]	250	-23.98
Gradient channel		
GPNAM 1	SMSQ10.100	
GPNAM 3	SMSQ10.100	
GPZ1 [%]	11.00	
GPZ3 [%]	30.00	
P16 [µsec]	1000.00	

Appendix S10. Pulse program for 3D H_M-C_MH_M SFNOESY-HMQC.

;stnoesyhmqc3d.Hm-LmHm: sotast 3D NUESY-HMQL for Hm(F1)-Lm(F2)Hm(F3) ;H(t1) -NOE -H-C(t2)-H(t3): H(t1) for methyl 1H, C(t2) and H(t3) are for methyl

;Youlin Xia on 05/05/2016 ;\$CLASS=HighRes ;\$DIM=3D :STYPE= ; \$SUBTYPE= ; \$COMMENT= prosol relations=<triple> #include <Avance.incl> #include <Delay.incl>
#include <Grad.incl> "d11=30m" "in0 =inf1/2" "in10=inf2/2" "d0=3u" "d10=0u" "d14=1s/(cnst4*2)" ;cnst4=125 "TAU=d8-p3-p16-0.5m" "p41=7.2/(cnst2*bf1/1000000)" /* PC9 pulse length */ "p42=4.875/(cnst2*bf1/1000000)" /* REBURP pulse length */ "spw25=plw1*(pow((p1/p41)/0.125,2))" "spoff25=0" /* PC9 offset */ "spw26=plw1*(pow((p1*2/p42)/0.0798,2))" /* PC9 offset */ "spoff26=0" "spw27=plw1*(pow((p1*(cnst3/90)/p41)/0.125,2))" "spoff27=bf1*(cnst1/1000000)-o1" /* PC9 offset */ "spw28=plw1*(pow((p1*2/p42)/0.0798,2))" /* PC9 offset */ "spoff28=spoff27" "DELTA1=d14-p16-d16-p41*0.5" "DELTA2=p41*0.5-de-4u" aqseq 321 1 d11 ze 2 d11 do:f2 3 d1 pl0:f1 pl2:f2 10u UNBLKGRAD ;(p3 ph1):f2 p16:gp3*1.2 200u "d21 = d10*2 - p3*0.637*2" "if (d21 < 0) { d21 = 0; }" :NOFSY 4u fq=cnst1(bf ppm):f1 # ifdef cpd 4u pl12:f2 5u cpds5:f2 (p41:sp25 ph3):f1 dØ dØ (p42:sp26 ph5):f1 (p41:sp25 ph4):f1 5u do:f2 4u pl2:f2 # else (p41:sp25 ph3):f1 d0 (p4 ph1):f2 dØ (p42:sp26 ph5):f1 3u (p4 ph1):f2 Зu (p41:sp25 ph4):f1 # endif 4u ;mixing TAU fq=0:f1 (p3 ph1):f2 p16:gp3 0.5m ;0 Hz offset

;HMQC2 (p41:sp27 ph1):f1 p16:gp1 d16 (center (p42:sp28 ph8):f1 (DELTA1 p3 ph6 d21 p3 ph7 DELTA1):f2) DELTA2 p16:gp1 d16 pl12:f2 4u BLKGRAD qo=2 ph31 cpd2:f2 d11 do:f2 mc #0 to 2 ;for topspin2.0 F1PH(rd10 & rp6 & ip3, id0) F2PH(ip6, id10) :for topspin3.0 ;F1PH(calph(ph3, +90), caldel(d0, +in0)) ;F2PH(calph(ph6, +90), caldel(d10, +in10)) exit ph1 =0 ph2 =1 ph3 =0 2 ph4 =0 ph5 =0 0 0 0 0 1 1 1 1 ph6 =0 0 2 2 ph7 =0 ph8 =0 ph31=0 2 2 0 2 0 0 2 ;pl1 : f1 channel - power level for pulse (default) ;pl2 : f2 channel - power level for pulse (default) ;pl2: f2 channel - power level for rhoped 90 degree pulse ;sp25: f1 channel - power level for shaped 90 degree pulse ;sp26: f1 channel - power level for shaped 180 degree pulse ;sp28: f1 channel - power level for shaped 90 degree pulse ;sp28: f1 channel - power level for shaped 180 degree pulse ;sp28: f1 channel - power level for shaped 180 degree pulse ;spana25: Reburp.1000 ;spnaa27: Reburp.1000 ispnam28: Reburg.lowe ispnam28: Reburg.lowe pl : f1 channel - 100 degree high power pulse p2 : f1 channel - 180 degree high power pulse p3 : f2 channel - 100 degree high power pulse p14 : f2 channel - 180 degree high power pulse p16: homespoilgradient pulse [1 msec] ;p41: f1 channel - 90 degree shaped pulse ;p41: f1 channel - 180 degree shaped pulse ;p42: f1 channel - 180 degree shaped pulse ;d0 : incremented delay (F1 in 30) ;d1 : relaxation delay; 1-5 * T1 [3 usec] :d2 : 1/((2J)XH) ;02 : 1/((2)/AN)
;d8 : mixing time
;d10: incremented delay (F2 in 30)
;d11: delay for disk I/0
;d14: 1/(2)CH) [3 usec] [30 msec] [3.7 msec] ;d14: 1/(2)CH)
;d15: elay for homospoll/gradient recovery
;d21: run time delay given by the pulse sequence
;cnst1: center of methyl 1H in ppm [0.9]
;cnst2: Hexcitation bandwidth [5]
;cnst3: 11CH of methyl [123]
;cnst4: 11CH of methyl [123]
;cnst5: 11CH of aromatic ring [160]
;c01: center of methyl 13C chemical shifts [16] ;inf1: 1/SW(H) = 2 * DW(H) ;inf2: 1/SW(X) = 2 * DW(X) ;in0: 1/(2 * SW(H)) = DW(H) ;nd0: 2 ;in10: 1/(2 * SW(X)) = DW(X) :nd10: 2 ;NS: 4 * n ;DS: 8 ;td1: number of experiments in F1
;td2: number of experiments in F2 :td2: number of experiments in F2 ;FnN00E: States-TPPI (or TPPI) in F1 ;FnN00E: States-TPPI (or TPPI) in F2 ;cp02: decoupling according to sequence defined by cpdprg2 ;cp02: f2 channel - 9 degree pulse for decoupling sequence ;cpdprg2: f2 channel - bi_garp_2pl ;for z-only gradients: ;gpz1: 25% ;gpz3: 30%

;use gradient files: ;gpnam1: SMSQ10.100 ;gpnam3: SMSQ10.100

Appendix S11. Parameter set for 3D H_M-C_MH_M NOESY-HMQC.

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Pulseprogram para	meters of datase	t:	p41 [µsec]	2116.90	
C:/NMR/sofast/220	/pdata/1		p42 [µsec]	1433.32	
			PLW0 [W, -dBW]	0	1000.00
General			PLW1 [W, -dBW]	12	-10.79
PULPROG	sfnoesyhmqc3d.	nm.HCH	SPNAM 25	Pc9 4 90.1000	
TD	1356		SPOAL25	0.500	
SWH [Hz, ppm]	13586.96	15.9789	spoffs25 [Hz]	0	
AQ [sec]	0.0499008		spw25 [W, -dBW]	0.036033	14.43
RG	90.5		SPNAM 26	Reburp.1000	
DW [µsec]	36.800		SPOAL26	0.500	
DE [µsec]	25.00		spoffs26 [Hz]	0	
CNST2	4.000000		spw26 [W, -dBW]	0.77141	1.13
CNST3	110.0000000		SPNAM 27	Pc9_4_90.1000	
CNST4	125.0000000		SPOAL27	0.500	
d0 [sec]	0.0000300		spoffs27 [Hz]	-3147.91	
D1 [sec]	0.2000000		spw27 [W, -dBW]	0.053827	12.69
D8 [sec]	0.3000001		SPNAM 28	Reburp.1000	
d10 [sec]	0		SPOAL28	0.500	
d11 [sec]	0.03000000		spoffs28 [Hz]	-3147.91	
d14 [sec]	0.00400000		spw28 [W, -dBW]	0.77141	1.13
D16 [sec]	0.00020000		Channel f2		
D21 [sec]	0.00180000		SFO2 [MHz]	213.8118466	
DELTA1 [sec]	0.00174155		O2 [Hz, ppm]	3741.64	17.500
DELTA2 [sec]	0.00102945		NUC2	13C	
DS	8		CPDPRG 2	bi_garp_2pl	
in0 [sec]	0.00016800		P3 [µsec]	11.20	
in10 [sec]	0.00011690		P4 [μsec]	22.40	
INF1 [µsec]	336.00		PCPD2 [µsec]	50.00	
INF2 [µsec]	233.80		PLW2 [W, -dBW]	180	-22.55
NS	8		PLW12 [W, -dBW]	9.0317	-9.56
P1 [μsec]	14.50		PLW30 [W, -dBW]	9.0317	-9.56
TAU [sec]	0.29848883		PLW31 [W, -dBW]	36.127	-15.58
ZGOPTNS			Gradient channel		
Channel fl			GPNAM 1	SMSQ10.100	
SFO1 [MHz]	850.3039982		GPNAM 3	SMSQ10.100	
01 [Hz, ppm]	3998.21	4.702	GPZ1 [%]	11.00	
NUC1	1H		GPZ3 [%]	30.00	
CNST1	1.0000000		P16 [µsec]	1000.00	

(1/2)

(2/2)

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