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Supporting Information

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Enhancing NMR Sensitivity of Natural-Abundance Low-γ Nuclei by Ultrafast Magic-Angle-Spinning Solid-State NMR Spectroscopy

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Experimental

Materials

Ibuprofen, anhydrous danazol (DNZ), anhydrous vanillin (VAN), and ethyl acetate were purchased from Sigma-Aldrich (St. Louis, MO, USA), LGM Pharma (Boca Raton, Florida), Fisher Scientific (Fair Lawn, New Jersey), and Acros Organics, respectively. All samples were used as received without further purification.

Preparation of co-crystals

The 1:1 danazol-vanillin cocrystal was prepared by the reaction crystallization method.[1] Stoichiometric amounts of cocrystal components (danazol and vanillin) were added to nearly saturated vanillin solution in ethyl acetate. The suspension was protected from light and stirred with a magnetic stir bar for 24 hours at room temperature. The suspension was then vacuum filtered.

Solid-State NMR Experiments

All solid-state NMR experiments were performed on an Agilent VNMRS 600 MHz solid-state NMR spectrometer under 60 kHz MAS using a triple-resonance 1.2 mm MAS probe (Agilent) operating at 599.8 MHz for ^{1}H and 150.8 MHz for ^{13}C. All the pulse sequences used in this study are shown in Figure 1. For the ^{13}C-detected experiments (Fig. 1a and 1b), a 90^{o} pulse was applied on the ^{1}H channel right after the final ^{13}C signal acquisition to flip the residual ^{1}H magnetization to the $+z$ axis to speed-up T_1 relaxation.[2–4] As ^{1}H magnetization loss due to polarization transfer is very little in natural-abundance sample, the number of CP contacts (i.e. N) in ^{13}C-detected MCP experiments is generally determined by the proton $T_{1\rho}$ relaxation during the CP spin-lock time and signal acquisition periods. Therefore, N could be easily optimized by measuring ^{1}H $T_{1\rho}$ relaxation behavior using the same RF field strength as that applied for CW decoupling (or CP spin-locking). However, it is difficult to optimize the number of CP contacts for the ^{1}H-detected MCP experiments (Fig. 1c) due to the poor sensitivity of ^{13}C signals. ^{13}C signal loss is generally

resulted from the $^{13}C \rightarrow {}^{1}H$ CP instead of $T_{1\rho}$ relaxation of ^{13}C as it is generally very long due to the weak ^{13}C-^{13}C dipolar couplings. Therefore, in the ^{1}H-detected MCP HETCOR experiment, the total contact time for $^{13}C \rightarrow {}^{1}H$ polarization transfer is set to be slightly smaller than the initial CP contact time for $^{1}H \rightarrow {}^{13}C$ polarization transfer. For the ^{1}H-detected MCP HETCOR experiment, HORROR[5] sequence is applied to remove residual proton magnetization after the initial $^{1}H \rightarrow {}^{13}C$ polarization transfer. In this study, the 90° pulse width was 2 μs on both ^{1}H and ^{13}C RF channels. Ramped DQ CP[6] was applied for all heteronuclear $^{1}H \rightarrow {}^{13}C$ and $^{13}C \rightarrow {}^{1}H$ polarization transfer with w_{1H}~14 kHz and w_{1C}~46 kHz. RF field strengths around 14 kHz and 17 kHz were applied for ^{1}H and ^{13}C heteronuclear decoupling during ^{13}C and ^{1}H chemical shift evolution, respectively. For ^{13}C-detected MCP HETCOR experiment, each CP contact time was 0.4ms, whereas in the ^{1}H-detected MCP HETCOR experiment, the initial $^{1}H \rightarrow {}^{13}C$ CP contact time was 2.0 ms, and each of $^{13}C \rightarrow {}^{1}H$ CP contact time was 0.4ms. For all the MCP based experiments, all the acquired FIDs within the same scan were added up to make a single FID before further 1D or 2D Fourier transformation. The ^{13}C Chemical shift was externally referenced to adamantane by setting the downfield ^{13}C resonance signal to 38.5ppm. [7]

Theoretical analysis

We consider an isolated ^{13}C-^{1}H (*S*-*I*) spin pair for the theoretical treatment. According to the multiple-contact cross polarization theory developed by Pines *et al*,[8] the low-γ nuclei magnetization obtained through *n*-th CP contact is given by

$$M_n = \frac{\gamma_I}{\gamma_S}(1-\xi)^n M_0 \qquad \text{(S1)}$$

where $\xi = \frac{S(S+1)N_s}{I(I+1)N_I}$. γ_I and γ_S are the gyromagnetic ratios of nuclei *I* and *S*, respectively. M_0 is the thermal equilibrium Boltzmann magnetization. N_S and N_I are the number of *S* and *I* spins, respectively.

If we take ^{1}H $T_{1\rho}$ relaxation into consideration, and ignore the ^{13}C $T_{1\rho}$ relaxation (because it is generally very long), we have

$$M_n = \frac{\gamma_I}{\gamma_S}(1-\xi)^n M_0 e^{\frac{-n(\tau_{cp}+\tau_{aq})+\tau_{aq}}{T_{1\rho}}} \tag{S2}$$

τ_{cp} and τ_{aq} are the time of each CP contact and signal acquisition, respectively. $T_{1\rho}$ is the ^{1}H spin-lattice relaxation time in the rotating frame.

Therefore, by summing up all the acquired ^{13}C signals in each CP contact, we can obtain

$$\begin{aligned} M_{total} &= \sum_{n=1}^{N} M_n = \sum_{n=1}^{N} \frac{\gamma_I}{\gamma_S}(1-\xi)^n M_0 e^{\frac{-n(\tau_{cp}+\tau_{aq})+\tau_{aq}}{T_{1\rho}}} \\ &= \frac{\gamma_I}{\gamma_S} M_0 \sum_{n=1}^{N} (1-\xi)^n e^{\frac{-n(\tau_{cp}+\tau_{aq})+\tau_{aq}}{T_{1\rho}}} \end{aligned} \tag{S3}$$

where N is the number of CP contacts in a MCP experiment.

Since in the ^{13}C natural abundant sample, $\xi << 1$

we have

$$(1-\xi)^n : e^{-n\xi} \tag{S4}$$

Therefore,

$$\begin{aligned} M_{total} &= \frac{\gamma_I}{\gamma_S} M_0 \sum_{n=1}^{N} e^{-n\xi} e^{-n(\tau_{cp}+\tau_{aq})/T_{1\rho}+\tau_{aq}/T_{1\rho}} = \frac{\gamma_I}{\gamma_S} M_0 e^{\tau_{aq}/T_{1\rho}} \sum_{n=1}^{N} e^{\frac{[-n(\xi+\frac{\tau_{cp}+\tau_{aq}}{T_{1\rho}})]}{T_{1\rho}}} \\ &= \frac{\gamma_I}{\gamma_S} M_0 e^{\tau_{aq}/T_{1\rho}} \frac{1-e^{\frac{[-N(\xi+\frac{\tau_{cp}+\tau_{aq}}{T_{1\rho}})]}{T_{1\rho}}}}{e^{\xi+\frac{\tau_{cp}+\tau_{aq}}{T_{1\rho}}}-1} \end{aligned} \tag{S5}$$

Assuming that the root-mean-square (rms) noise signal amplitude is constant in each signal acquisition period, $M_{noise,}$ the signal-to-noise (S/N) ratio enhancement obtained by MCP in comparison to single-contact CP is,

$$\eta = (\frac{M_{total}}{N^{1/2}M_{noise}}) / (\frac{M_1}{M_{noise}}) = \frac{e^{(\tau_{aq}+\tau_{cp})/T_{1\rho}}[1-e^{-N(\xi+\frac{\tau_{aq}+\tau_{cp}}{T_{1\rho}})}]}{N^{1/2}(1-\xi)(e^{\xi+(\tau_{aq}+\tau_{cp})/T_{1\rho}}-1)} \quad \text{(S6)}$$

For the natural abundant sample, we have $\xi \to 0$

If the ^{1}H $T_{1\rho}$ is long compared to the total CP contact time and signal acquisition time, then

$$\eta \approx \frac{1-(1-N\xi)}{N^{1/2}\xi(1-\xi)} = \frac{N^{1/2}}{1-\xi} \approx N^{1/2} \quad \text{(S7)}$$

Here the overall *S*/*N* ratio obtained from MCP is roughly the same as that from the conventional single-contact CP experiment with *N* times the number of scans, assuming that the proton $T_{1\rho}$ relaxation could be ignored.

As is also shown in Eq.(S5), if the $T_{1\rho}$ is short, with the increase in the number of CP contacts, η will decrease, as the term $e^{-N(\xi+\frac{\tau_{aq}+\tau_{cp}}{T_{1\rho}})}$ will play a significant role.

For the conventional 2D ^{1}H-detected ^{1}H/^{13}C HETCOR NMR experiment, the *S*/*N* ratio enhancement in comparison to the regular ^{13}C-detected HETCOR experiment is, [9]

$$\varsigma = (f^2 d)^{1/2}(\frac{\gamma_H}{\gamma_C})^{3/2}(\frac{w_C}{w_H})^{1/2}(\frac{Q_H}{Q_C})^{1/2}\frac{A_H}{A_C} \quad \text{(S8)}$$

where Q is the quality factor of the sample coil; f is the polarization efficiency between ^{13}C and ^{1}H spins; d is the receiver duty factor for ^{1}H detection; and A includes the effects of coil geometry, filling factor, receiver noise etc. w_C and w_H are the effective line-widths for ^{13}C and ^{1}H peaks, respectively. Typically, $A_H/A_c \approx 1$, $Q_H/Q_c \approx 2$, d=1, f=0.5, γ_H/γ_C=3.98;[9] thus,

$$\varepsilon = 5.6(\frac{w_C}{w_H})^{1/2} \quad \text{(S9)}$$

For the ^{1}H-detected MCP HETCOR experiment with N ^{13}C→^{1}H CP contacts, the enhancement factor can be written as

$$\varepsilon = 5.6N^{1/2}(\frac{w_C}{w_H})^{1/2} \quad \text{(S10)}$$

Therefore, ^{1}H-detected MCP HETCOR spectrum can render a signal enhancement of $N^{1/2}$ compared to the regular ^{1}H-detected HETCOR spectrum. However, in the ^{1}H-detected MCP HETCOR experiment, the number of inverse ^{13}C→^{1}H CP contacts (i.e. N) is limited by the initial ^{1}H—>^{13}C CP contact time as well as each ^{13}C→^{1}H CP contact time. In contrast, N can be as large as possible as long as there is ^{1}H proton magnetization remaining in the transverse plane for polarization transfer in the ^{13}C-detected MCP HETCOR experiment. However, a large N also means a long CW spin-locking time, which may result in widespread magnetization exchanges (i.e. due to proton spin diffusion) among protons, and thus multiple remote ^{1}H-^{13}C cross peaks. Assuming that the proton spin diffusion could be ignored (such as the heavily deuterated biological samples), when η is larger than ε, the ^{13}C-detected MCP HETCOR experiment can provide a higher S/N ratio compared to the ^{1}H-detected MCP HETCOR experiment.

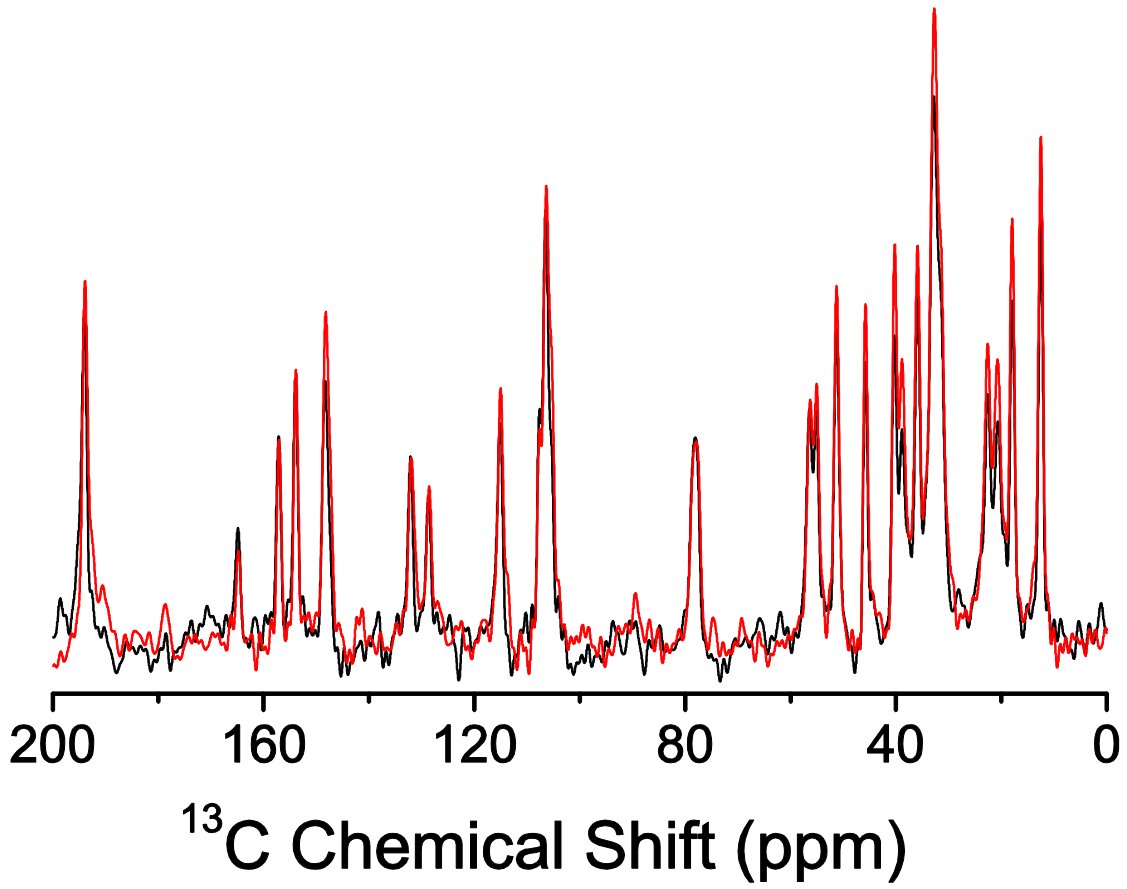

Figure S1. Carbon-13 CPMAS spectra of danazol/vanillin co-crystals with (red) and without (black) the ^{1}H 90^{o} flip pulse obtained using $MCP_{N=16}$.

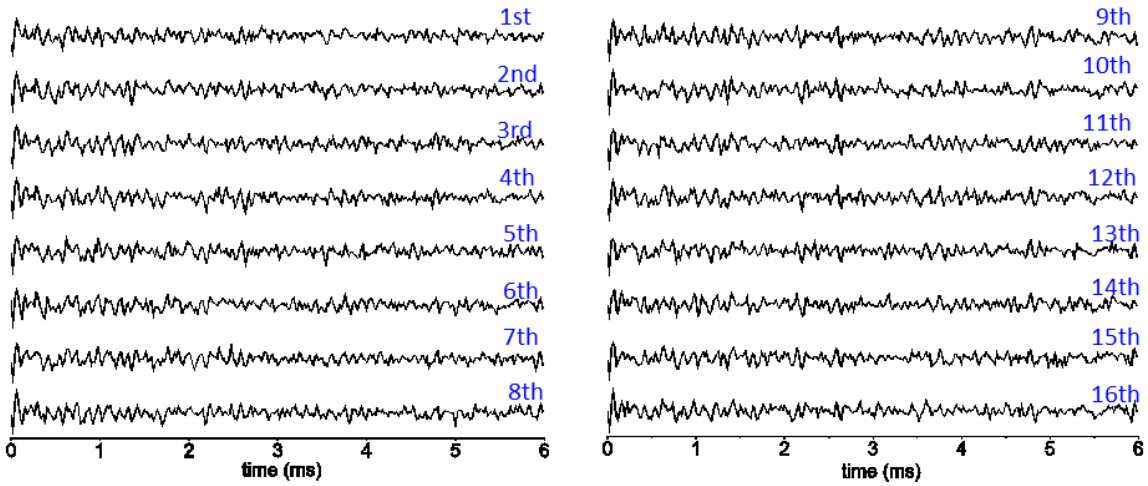

Figure S2. Carbon-13 CPMAS FIDs of danazol/vanillin co-crystals using $MCP_{N=16}$. As is clearly shown, due to the long proton $T_{1\rho}$ of this cocrystal, the signal intensities observed for successive FIDs are similar.

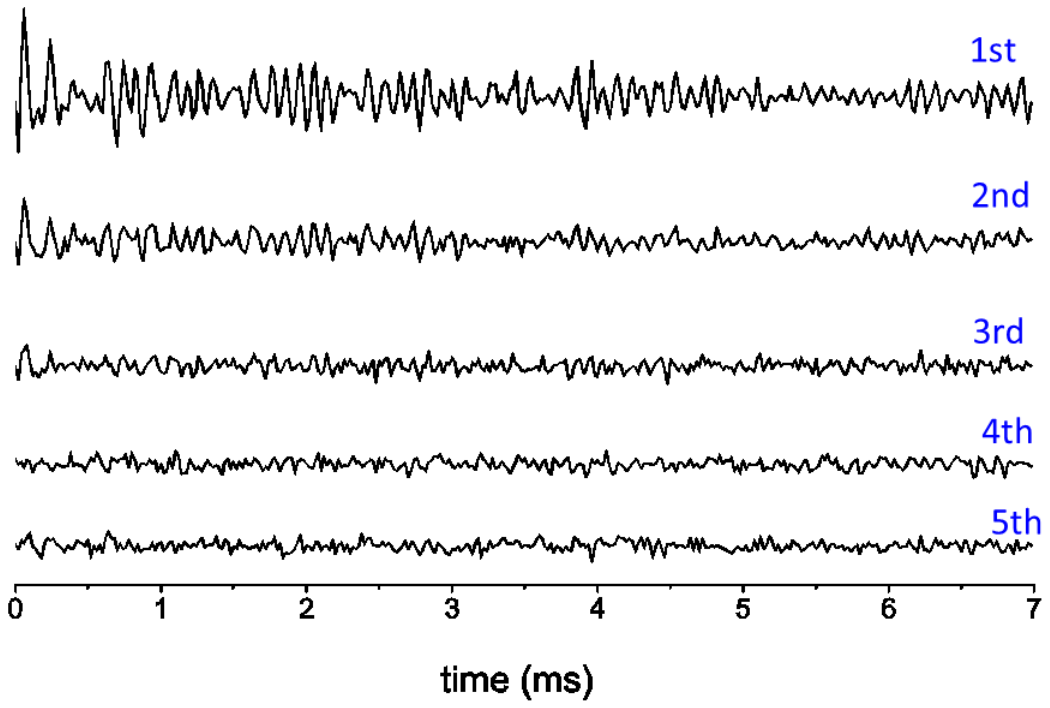

Figure S3. Carbon-13 CPMAS FIDs of ibuprofen using $MCP_{N=5}$. As is clearly shown, due to the short proton $T_{1\rho}$ of ibuprofen, a significant signal decrease can be observed, and there is basically less signal observed in the forth and fifth FIDs.

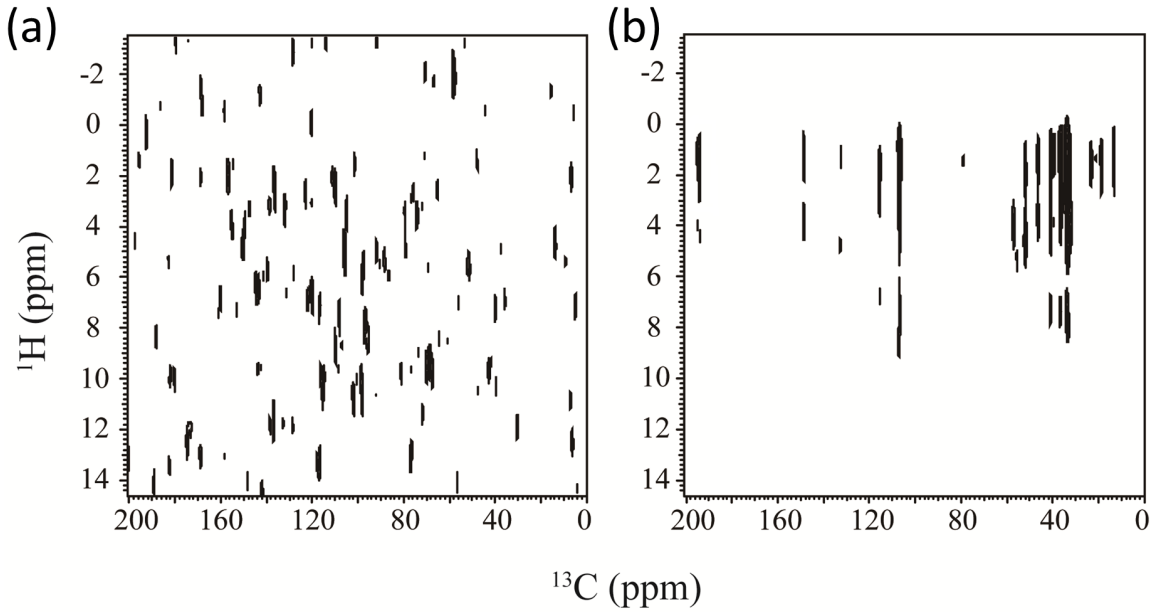

Figure S4. ^{13}C-detected 2D ^{1}H/^{13}C HETCOR spectra of danazol/vanillin co-crystals obtained using the pulse sequence shown in Fig. 1b with N=1(a) and N=20(b), respectively. The intensities of Fig. S4a were scaled by a factor of 10. For both experiments, each CP contact time was 0.4ms and the signal acquisition time was 6ms. The recycle delay was 7s and the number of scans for each t_1 increment was 200.

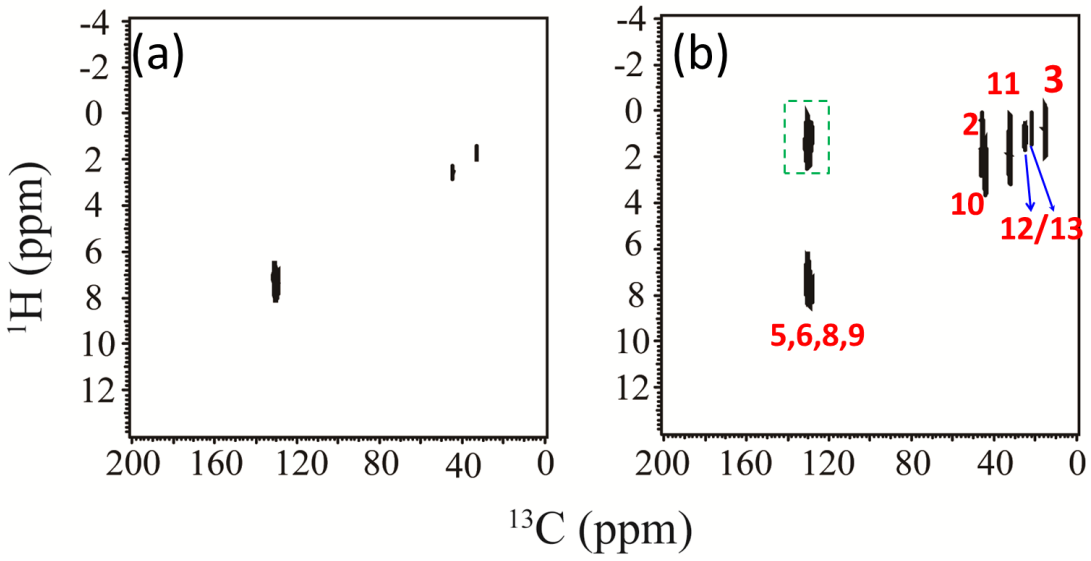

Figure S5. ^{13}C-detected 2D ^{1}H/^{13}C HETCOR spectra of ibuprofen obtained using the pulse sequence shown in Fig. 1b with N=1(a) and N=4(b), respectively. Each CP contact time was 0.4ms and each signal acquisition time was 7ms. The recycle delay was 3s and the number of scans for each t_1 increment was 136. The correlation between aromatic carbon and methyl proton is indicated with a green dash rectangle.

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