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Ultrafast ^1H MAS NMR Crystallography For Natural Abundance Pharmaceutical Compounds

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Abstract

Magic angle spinning (MAS) NMR is a powerful method for the study of pharmaceutical compounds, and probes with spinning frequencies above 100 kHz enable atomic-resolution analysis of sub-micromole quantities of fully protonated solids. Here we present an ultrafast NMR crystallography approach for structural characterization of organic solids at MAS frequencies of 100–111 kHz. We assess the efficiency of ^1H -detected experiments in the solid state and demonstrate the utility of 2D and 3D homo- and heteronuclear correlation spectra for resonance assignments. These experiments are demonstrated for an amino acid, U- ^{13}C , ^{15}N histidine and also for the significantly larger, natural product Posaconazole, an anti-fungal compound investigated at natural abundance. Our results illustrate the power for characterizing organic molecules, enabled by exploiting the increased ^1H resolution and sensitivity at MAS frequencies above 100 kHz.

Graphical Abstract

SUPPORTING INFORMATION

Supporting information is available free of charge from the ACS website:

- Posaconazole T_1 data, efficiency of SPC53 triple quantum experiments, histidine T_2 measurements, constant time SPC53 DQ-SQ correlation experiment, DFT calculated vs solution NMR chemical shifts, tables of ^{13}C and ^1H solid state NMR and DFT chemical shifts

Keywords

Ultrafast magic angle spinning; proton detection; NMR crystallography; Posaconazole; pharmaceuticals

INTRODUCTION

Solid state nuclear magnetic resonance (SSNMR) spectroscopy is ubiquitously employed for the characterization of solid-phase pharmaceuticals, both pure molecules and complex formulations^{1–3}. The span of SSNMR applications of pharmaceuticals varies from the identification of polymorphs and phase transitions to the assessment of formulation stability and homogeneity^{4–7}. One of the greatest strengths of SSNMR for the analysis of pharmaceutical products is that long-range order, such as present in crystals, is not required.

Most commonly, solid-state NMR experiments are performed with magic angle spinning (MAS), to achieve narrow lines and the accompanying increased sensitivity^{8–10}. At MAS frequencies of ~ 60 kHz, attainable by a number of MAS NMR probes used by SSNMR practitioners today, heteronuclear dipolar and CSA interactions are efficiently averaged out. In contrast, homonuclear ¹H-¹H dipolar couplings are not averaged at these MAS frequencies, and highly resolved ¹H-detected spectra cannot be generally acquired in fully protonated samples. This impediment can be alleviated by using extensively deuterated samples, which yield narrow ¹H lines^{11–13}. Unfortunately, deuteration is not a practical approach for many systems, including pharmaceutical formulations, since it involves additional synthesis.

The recent development of probes that can deliver MAS frequencies above 100 kHz has revolutionized the field of MAS NMR^{14–16}. At these MAS frequencies, significant gains in both resolution and sensitivity are observed because ¹H-¹H dipolar interaction can be efficiently averaged out by a combination of spinning and homonuclear decoupling using radiofrequency (rf) pulses^{17–19}. This regime, called “ultrafast MAS”, enabled the implementation of a broad range of ¹H-detected experiments for resonance assignments and structural characterization of fully protonated systems, including ¹H-¹H multidimensional correlation experiments²⁰. Such experiments are powerful for analyzing organic solids since ¹H chemical shifts provide valuable information on the molecular and supramolecular structure, not easily available from the chemical shifts of heteronuclei, such as ¹³C and ¹⁵N²¹. Furthermore, when combined with quantum chemical calculations of NMR parameters, they permit to extract information about supramolecular organization, such as the crystal lattice, in an approach termed “NMR crystallography”^{22–25}.

Here we use the NMR crystallography approach for the structural characterization of two molecules at an MAS frequency of 111 kHz. We examined the performance of several 2D and 3D experiments for crystalline U-¹³C, ¹⁵N histidine. Specifically, we assessed different double quantum excitation schemes, including constant time variants²⁶, in homonuclear chemical shift correlation experiments and determined the optimum conditions for scalar coupling-based ¹³C-¹H correlation experiments. In addition, we also applied this approach to Posaconazole, a 700 Da antifungal agent²⁷. Using both examples, the optimum set of

experiments for homonuclear (^1H - ^1H) and heteronuclear ($^{13}\text{C}/^{15}\text{N}$ - ^1H) ^1H -detected correlation spectroscopy is discussed. In addition, we demonstrate that quantum chemical calculations of ^1H , ^{13}C , and ^{15}N chemical shifts provide invaluable information, including identification of intra- and intermolecular correlations, and permit spectral assignments.

MATERIALS AND METHODS

Samples

U- ^{13}C , ^{15}N histidine was purchased from Cambridge Isotope Laboratories and used without further purification. Posaconazole was kindly provided by Dr. Yongchau Su (Merck). Samples were packed into 0.7 mm rotors. Each rotor contained less than 1 mg of sample in 0.5 μL available sample volume.

MAS NMR Spectroscopy

MAS NMR experiments were collected on a 16.4 T Bruker Avance III standard bore spectrometer, outfitted with a Bruker 0.7 mm HCN MAS probe. The MAS frequency was 111 kHz, controlled to within ± 25 Hz with a Bruker MAS III controller. Heteronuclear multiple quantum coherence (HMQC) 2D correlation spectra and double quantum (DQ) 1D spectra on histidine were also acquired on a 11.7 T Bruker Avance NEO wide bore spectrometer outfitted with a Bruker 0.7 mm HCN MAS probe. Typical 90° pulse lengths were 0.7 μs (^1H), 1.5 μs (^{13}C), and 2.4 μs (^{15}N).

All experiments on U- ^{13}C , ^{15}N histidine used a pulse delay of 6 s and 10 kHz WALTZ16 ^{13}C and ^{15}N decoupling during ^1H acquisition. ^1H 1Ds were acquired with a Hahn echo sequence to avoid dead time and ^1H background.

To evaluate the performance of DQ excitation under ultrafast MAS, a number of conditions were tested for three different DQ excitation schemes. SPC-5 $_3$ ^{28,29} ^1H DQ measurements used 368 kHz rf field and optimized excitation and reconversion times of 86.49 μs and 108.11 μs , respectively. An average single value of 97 μs was chosen for the excitation and reconversion periods in the 2D experiments. R14 $_4$ ⁵-symmetry based³⁰ DQ measurements require a 194 kHz B_1 -field for excitation and reconversion at 111 kHz MAS, with the optimum time being 102.96 μs for both excitation and reconversion. BABA³¹ ^1H DQ experiment used 357 kHz excitation pulses of 0.7 μs duration and a 0.5 μs z-filter, with 4 rotor periods for BABA DQ excitation (36.036 μs DQ evolution time) to attain optimal performance. 128 points were acquired in the t_1 dimension (11.5 ms) and for each t_1 point 16 transients were averaged.

^1H - ^1H fpRFDR 2D spectra were acquired over a range of mixing times from 67.5 μs to 1 ms. 512 points were acquired in the t_1 dimension (13.8 ms); 16 transients were averaged. The ^1H - ^1H RFDR DQ-SQ 3D experiment was performed with a 36 μs DQ excitation time and 125 μs RFDR mixing. 32 points were acquired in each indirect dimension (0.6 ms); 16 transients were averaged. The 2D ^{13}C - ^1H HETCOR spectrum was acquired with random exponentially biased (2.5 ms) non-uniform sampling (NUS) to 25%; the sampling schedule was generated in Topspin using the automatic sampling scheduling tool. For cross polarization (CP), the rf fields were 148.75 kHz and 27.75 kHz on ^1H and ^{13}C , respectively

and a 10% linear ramp was applied on ^1H . The contact times were 1 ms and 0.8 ms for out and back transfers, respectively. 128 points were acquired in the t_1 dimension (2.3 ms); 16 transients were averaged. The 2D ^{15}N - ^1H HETCOR utilized 77 kHz on ^1H with a 30% ramp, and 34 kHz on ^{15}N for CP. Contact times were 2 ms out and 0.4 ms back. 236 points were acquired in the t_1 dimension (4.2 ms); 4 transients were averaged.

To evaluate the efficiency of ^{13}C - ^1H heteronuclear multiple quantum coherence (HMQC) and heteronuclear single quantum coherence (HSQC) scalar transfers, several evolution times were tested: 1.43 ms to 4.167 ms (120 Hz to 350 Hz J coupling) for HMQC, and 0.714 ms to 1.667 ms (150 Hz to 350 Hz J coupling) for HSQC. The ^{13}C - ^1H HMQC 2D used 2.5 ms (200 Hz) J evolution. 128 points were acquired in the t_1 dimension (1.2 ms); 64 transients were added up for each FID. The ^{15}N - ^1H HSQC 2D also used 2.5 ms (200 Hz) J evolution. 256 points were acquired in the t_1 dimension (4.6 ms); 16 transients were averaged.

All experiments on Posaconazole used a recycle delay of 10 s (considering an average ^1H T_1 of 5 s, Figure S1). For the ^{13}C - ^1H HETCOR experiment, the rf fields during the CP were 148.75 kHz on ^1H with a 10% ramp, and 27.75 kHz on ^{13}C . The contact times were 1 ms and 0.8 ms for out and back CP transfers, respectively. 256 points were acquired in the t_1 dimension (2.3 ms); 64 transients were accumulated, for a total experiment time of 45 hours. The ^{13}C - ^1H HMQC 2D used 2 ms (250 Hz) J evolution. 128 points were acquired in the t_1 dimension (1.2 ms); 128 transients were averaged, for a total experiment time of 45 hours.

^1H - ^1H fpRFDR 2D spectra were acquired at three mixing times: 90 μs , 200 μs , and 1 ms. 512 points were acquired in the t_1 dimension (13.8 ms); 16 transients were averaged, for a total experiment time of 23 hours per spectrum. The ^1H - ^1H RFDR DQ-SQ 3D experiments were performed using a 27 μs DQ excitation time and 150 μs RFDR mixing, with $\{\text{XY4}\}_4^1$ phase cycling. 48 points were acquired in each indirect dimension (0.8 ms); 16 scans were averaged, for a total experiment time of 102 hours.

All spectra were processed in Topspin without apodization for 1Ds and 30° or 60° sine squared or Lorentzian-to-Gaussian apodization for 2Ds. The processed data were analyzed in Sparky³² or CCPNMR^{33,34}.

Density Functional Theory (DFT) Calculations

All DFT calculations were performed using the Gaussian09³⁵ programs. All calculations were carried out for a four molecule cluster of Posaconazole in the crystal (CCDC database identifier YIMVUO³⁶). The all-atom geometry optimization was carried out using Gaussian09, at the B3LYP/6-31G level. Magnetic shielding tensor calculations were performed with the gauge-including atomic orbitals (GIAO) formalism³⁷⁻⁴⁰, as implemented in Gaussian09. The NMR chemical shift ab-initio calculation was carried out at the B3LYP/cc-pVTZ level. Experimental ^{13}C and ^1H isotropic chemical shifts were referenced to TMS and ^{15}N isotropic chemical shifts were referenced by converting isotropic magnetic shieldings σ_{iso} into chemical shifts using the relation $\delta_{\text{iso}} = \sigma_{\text{ref}} - \sigma_{\text{iso}}$, with the value of σ_{ref} determined by linear regression between calculated and experimental shifts.

RESULTS AND DISCUSSION

^1H - ^1H 2D Correlation Experiments

To test the performance of various ^1H -based experiments, a series of 2D and 3D through-space and through-bond correlation spectra were acquired on U- ^{13}C , ^{15}N histidine. We first evaluated the efficiency of ^1H - ^1H homonuclear double quantum (DQ) experiments. Multiple quantum correlations can provide valuable information through enhanced resolution or spectral editing⁴¹. The SPC-5₃, R14₄⁵, and BABA DQ excitation schemes tested are schematically illustrated in Figure 1A. The required ^1H field strengths for these measurements range from 194 kHz to 390 kHz at the MAS frequency of 111 kHz, which can be readily achieved with a 0.7 mm probe.

For the SPC-5₃ ^1H DQ experiment^{28,29}, the optimized DQ excitation and reconversion times were 86.49 μs and 108.11 μs , with an average and maximum DQ yield of 38% and 43%, respectively (Figure 1B, magenta). For the triple-quantum (TQ) SPC-5₃ experiment, the average TQ yield was 11%, using a single optimized excitation and reconversion time of 184 μs (Figure S2). The single optimized DQ excitation and reconversion time for the R14₄⁵ DQ experiment³⁰ was 102.96 μs , with an average and maximum DQ yield of 38% and 41%, respectively (Figure 1B, blue). Back-to-back (BABA) homonuclear DQ recoupling³¹ (Figure 1B, pink) resulted in average and maximum DQ yields of 28%, and 32.5%, respectively and had poor performance for sites with short T_2 s despite a short, 36.04 μs recoupling duration (Hahn echo T_2 decays shown in Figure S3).

While there is little overall difference in the performance of SPC-5₃ vs R14₄⁵, R14₄⁵ has lower rf field requirements, while the SPC-5₃ sequence appears to have a slightly better excitation bandwidth, as suggested by the slightly higher intensity of the downfield ^1H resonances. Therefore, we used the SPC-5₃ sequence in multidimensional experiments. While it is generally anticipated that a constant time (CT) variation during the DQ evolution period²⁶ improves ^1H resolution, CT requires a long and fairly uniform transverse DQ spin relaxation, a condition hard to meet with only few exceptions. In the case of histidine, the short T_2 relaxation times (Figure S3) caused loss of several DQ-SQ correlations (Figure S4). Therefore, the CT version of the experiment was not used for our studies of Posaconazole.

To establish through-space ^1H - ^1H connectivities, a 2D DQ-SQ ^1H - ^1H spectrum was acquired with SPC-5₃ excitation using 97 μs excitation and reconversion. As shown in Figure 2A (pink spectrum), many well-resolved resonances are obtained and can be readily assigned.

We also acquired a series of ZQ ^1H - ^1H correlation spectra with fpRFDR mixing⁴², using the {XY4}₄¹ phase cycling scheme⁴³ for detection of through-space ^1H - ^1H connectivities. For the small molecule histidine, outstanding resolution and efficient ^1H - ^1H mixing are obtained at a mixing time of 500 μs , as shown in Figure 2A (grey spectrum). For the larger molecule Posaconazole, we found that it is helpful for assignments to acquire spectra at several mixing times, as discussed below.

¹H-Detected 2D Heteronuclear Correlation Experiments

¹⁵N-¹H and ¹³C-¹H 2D correlation spectra for U-¹³C,¹⁵N histidine employ scalar-based and dipolar-based transfers in HMQC/HSQC and HETCOR experiments, respectively. As illustrated in Figure 2A, these experiments yield complementary information: the cross peaks in ¹⁵N-¹H HSQC and ¹³C-¹H HMQC spectra arise from single-bond correlations, while those in the HETCOR spectra are also associated with long-range through-space correlations. Therefore, a full set of experiments is required for resonance assignments and extracting distance information. Indeed, we were able to assign all resonances using a combination of 2D ¹³C-¹H and ¹⁵N-¹H HMQC/HSQC spectra, and long-range correlations are extracted from the HETCOR spectra. It is worth noting that for histidine and generally small molecules, long-range correlations are present with using CP contact times for out/back transfers of 1/0.8 ms (¹H-¹³C) and 2/0.4 ms (¹H-¹⁵N). Remarkably, the dipolar-based ¹H-¹H, ¹³C-¹H, and ¹⁵N-¹H spectra of histidine also contain cross-peaks corresponding to correlations between histidine signals and those of a crystallographic water molecule. Observation of these cross peaks was possible due to the rigid nature of this water molecule.

Peak intensities for the Ce1-He1 cross peak, extracted from the 1D traces through the direct dimensions of the HMQC and HSQC spectra for different evolution times are shown in Figure 2B. As can be appreciated, the efficiency of magnetization transfers is substantially higher at longer J-evolution time in the HMQC compared to the HSQC transfer. Furthermore, the ¹H linewidths in the HMQC experiment are independent of the scalar evolution time: the linewidths are 279 and 276 Hz at 1.43 ms (350 Hz) and 3.33 ms (150 Hz), respectively. In contrast, in the HSQC experiment, broader lines are observed at longer scalar evolution times: 243 and 269 Hz at 0.714 ms (350 Hz) and 1.67 ms (150 Hz), respectively. Thus, the HMQC sequence is superior for small organic solids and pharmaceutical compounds in powder formulations.

¹H-Detected 3D Homonuclear Correlation Experiments

While for small molecules, such as histidine, a combination of 2D heteronuclear correlation experiments is sufficient for complete resonance assignments, this is not the case for larger systems with potential resonance overlap. For more complex molecules, additional frequency dimensions are required. We therefore examined the performance of a 3D ¹H-¹H-¹H correlation experiment, where the RFDR recoupling is combined with SPC5₃ double-quantum-to-single-quantum transfer (DQSQ)^{14,20,28,29}. The pulse sequence for a RFDR-DQSQ correlation spectrum and representative 2D planes extracted from the 3D spectrum recorded for U-¹³C,¹⁵N-histidine are displayed in Figure 3A and 3B, respectively. Numerous well-resolved cross peaks are observed in the spectrum and were readily assigned.

Applications to Natural Abundance Pharmaceuticals: Posaconazole

We also applied ¹H-detected 2D and 3D homo- and heteronuclear experiments to the anti-fungal pharmaceutical compound Posaconazole. This natural product serves as an example for a larger, complex pharmaceutical. For resonance assignments of Posaconazole a series of 2D and 3D ¹H-¹H and ¹³C-¹H correlation spectra were acquired (Figure 4). Since the sample is not isotopically labeled, inherently low sensitivity is observed in the ¹³C-¹H HMQC and

HETCOR spectra, and longer range ^{13}C - ^1H correlations are not as readily observed. Therefore, fewer connectivities are present compared to the spectrum of U- ^{13}C , ^{15}N histidine acquired with the same CP contact time.

To assist with the resonance assignments of Posaconazole, NMR parameters were calculated using DFT for a four-molecule cluster extracted from a recent X-ray crystal structure of Posaconazole³⁶ (Figure 5A). By combining the DFT calculations with our experimental data, most resonances were readily assigned. The calculated ^{13}C chemical shifts are in excellent agreement with the solution NMR values reported previously⁴⁴ (Figure S5). The correlation between calculated and experimental ^{13}C chemical shifts is shown in Figure 5B. While overall agreement between experimental and DFT-calculated ^1H chemical shifts is good, several averaged DFT ^1H shifts exhibited deviations of up to several ppm from the experimentally determined values (Figure 5C), although the calculated ^1H chemical shifts agree well with another recent DFT study of Posaconazole⁴⁵. ^1H shifts that exhibit significant differences between averaged DFT ^1H shifts and experimental ^1H shifts include the triazolone group (H44). Inspection of the 4-molecule cluster used for DFT and resulting chemical shifts (Table S2) indicate good agreement with experimental ^1H shifts when an intermolecular hydrogen bond is formed with a neighboring molecule of Posaconazole (Figure 5D). In addition, notable shift differences between the experimental solution ^1H chemical shifts⁴⁴ and the solid state isotropic shifts determined here are also present (Figure 5C; bottom).

Since ^1H chemical shifts are very sensitive to intermolecular interactions⁴⁶, they are significantly influenced by hydrogen bonding in the crystal. Indeed, in recent room-temperature MAS NMR⁴⁷ and dynamic nuclear polarization (DNP) studies⁴⁸ of amorphous solid dispersions (ASD) of Posaconazole, different ^1H shifts from those observed here for the pure molecule are seen, underscoring the high sensitivity of ^1H chemical shifts to critical structural characteristics of molecular state and/or formulation. All calculated and solid state NMR chemical shifts are listed in Tables S1 (^{13}C , ^{15}N) and S2 (^1H), along with the previously reported solution NMR chemical shifts⁴⁴. In total, from the spectra presented above we assigned 27 of 28 ^1H and 27 out of 37 ^{13}C resonances. Unassigned carbon resonances are those without attached hydrogens.

Additional structural information can be extracted from a series of ^1H - ^1H 2D fpRFDR spectra at different mixing times. At short mixing times, only spatially close atoms will yield cross peaks, while at longer mixing times longer range correlations are seen. At the shortest ^1H - ^1H mixing time of 90 μs , only correlations for distances of $\sim 2\text{-}4\text{ \AA}$ are observed, while at the longest mixing time of 1 ms correlations corresponding to up to distances of $\sim 4\text{-}6\text{ \AA}$ are present. Several intra- and intermolecular correlations and the corresponding distances in the crystal structure for H50 are illustrated in Figure 6. These correlations are in excellent agreement with distances in the recent crystal structure of Posaconazole³⁶. As an aside, we note that, the 2D RFDR spectra of Posaconazole exhibit limited resolution and complete assignment, solely based on these spectra, was not possible. Therefore, the 3D ^1H - ^1H - ^1H DQ-SQ-RFDR experiment and DFT NMR calculation were particularly helpful for reliable ^1H chemical shift assignment. A major advantage of the ^1H - ^1H - ^1H 3D experiment over spectra that contain a ^{13}C dimension (e.g. ^{13}C - ^1H - ^1H) is the significant time saving for

natural abundance materials. The 2D ^{13}C - ^1H HETCOR experiment of Posaconazole required 45 hours of signal averaging (Figure 4), and the 3D ^1H - ^1H - ^1H spectrum was recorded in 102 hours. A 3D experiment with a ^{13}C dimension (e.g. ^{13}C - ^1H - ^1H) could not be acquired in a reasonable amount of time without implementing time-saving methods such as non-uniform sampling. Taken together, our results also underscore the benefits of combining several 2D and 3D ^1H -detected experiments for in-depth structural characterization of pharmaceutical compounds at natural abundance. Double-quantum based spectra provide additional information complementary to the single-quantum data sets. While the optimal set of measurements is determined by the system of interest, the general approach presented here can be adapted to a broad range of pharmaceutical solids.

CONCLUSIONS

Here we reported on applications of ultrafast ^1H MAS NMR to small molecules and in particular to the anti-fungal drug Posaconazole. Our work demonstrates that ultrafast MAS NMR spectroscopy combined with DFT calculations in ‘NMR crystallography’ is a powerful approach for structural characterization of pharmaceutical compounds at natural abundance with micromole quantities. We envision that applications of ultrafast MAS methods will extend to a wide range of pharmaceutical formulations, for probing atomic-level details of structure, polymorphism, and dynamics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(A) General pulse sequence for double quantum (DQ) correlation experiments: DQ excitation, followed by evolution in the indirect dimension, then reversion. The delay t_d is an optional dephasing delay for the removal of any residual transverse magnetization. Elements for different 3 DQ recoupling sequences are also shown. (B) Comparison of resonance intensities for the various ^1H homonuclear double quantum recoupling schemes for $\text{U-}^{13}\text{C}$, ^{15}N histidine, relative to a Hahn echo spectrum (black): SPC-5₃ (purple) 368 kHz recoupling, R14₄⁵ (blue) 194 kHz recoupling, BABA (pink) 357kHz recoupling. All spectra were acquired as 1-dimensional experiments at a MAS frequency of 111 kHz.

Figure 2.

(A) Through-space and through-bond ^1H -detected correlation experiments for $\text{U-}^{13}\text{C}, ^{15}\text{N}$ histidine at 111 kHz MAS. Full resonance assignments could be completed with this set of $^{13}\text{C}-^1\text{H}$, $^{15}\text{N}-^1\text{H}$, and $^1\text{H}-^1\text{H}$ spectra. The RFDR mixing time was 500 μs and contact times were 1 ms out, 0.8 ms back for $^1\text{H}-^{13}\text{C}$ CP, and 2 ms out, 0.4 ms back for $^1\text{H}-^{15}\text{N}$ CP. Both $^{13}\text{C}-^1\text{H}$ HMQC and $^{15}\text{N}-^1\text{H}$ HSQC used 200 Hz J evolution. Other experimental parameters are provided in the Materials and Methods section. (B) Efficiency of $^{13}\text{C}-^1\text{H}$ HSQC and HMQC correlations at selected evolution times. (C) 3D structure of histidine. Carbon atoms are colored gray, nitrogen atoms blue, oxygen atoms red, and hydrogen atoms white.

Figure 3. (A) Pulse sequence of and (B) 2D planes extracted from the 3D ^1H - ^1H - ^1H RFDR-DQSQ (SPC5₃) experiment of U- ^{13}C , ^{15}N -histidine. The chemical shift in the indirect ^1H DQ dimension is indicated in the lower right corner of each panel.

Figure 4.

(A) Blue (top): through-bond (HMQC) and through-space (HETCOR) ^{13}C - ^1H correlation spectra of Posaconazole acquired at 111 kHz MAS. The contact times were 1 ms out and 0.8 ms back for ^1H - ^{13}C CP. 250 Hz J evolution was used for the ^{13}C - ^1H HMQC. Gray (bottom) ^1H - ^1H 2D fpRFDR correlation spectra for three different mixing times: 90 μs (black), 200 μs (medium gray), and 1 ms (light gray) and slices from a 3D RFDR-DQSQ (SPC-5₃) correlation experiment. The chemical shift of the DQ dimension is indicated in the lower left of each panel. The apparent lower resolution of the HMQC relative to the HETCOR is due

to the lower sensitivity of the HMQC spectrum, requiring both an increase in the number of scans (128 vs. 64) and shorter sampling time in the indirect dimension (1.2 vs. 2.3 ms) for the same total experiment time. (B) Molecular structure of Posaconazole.

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Figure 5.

(A) DFT optimized cluster of four molecules of crystalline Posaconazole (CCDC database identifier YIMVUO). (B) (top) Correlation between DFT-calculated and experimental solid state ^{13}C chemical shifts; (bottom) correlation between solution and experimental solid state ^{13}C isotropic chemical shifts for Posaconazole. (C) (top) Correlation between DFT-calculated and experimental solid state 1H chemical shifts; (bottom) correlation between experimental solution and solid-state NMR 1H isotropic chemical shifts of Posaconazole. Best fits are shown as dotted lines and $y = x$ as a solid black line. All calculations were

performed with the Gaussian suite of programs. (D) Illustration of the intermolecular hydrogen bond between H44 of molecule 4 and O41 of molecule 3 in the crystal. The experimental and calculated ^1H chemical shifts for H44 are listed at the bottom. Due to the small size of the DFT cluster, only molecule 4 in the cluster exhibits this hydrogen bond, resulting in better agreement with solid state NMR shift than the average over the 4 molecules.

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Figure 6.

A) Selected region of the 2D ^1H - ^1H RFDR correlation spectra for two different mixing times. Several intra- and intermolecular correlations for H50 labeled (only a single ^1H resonance is observed for the 3 protons of this methyl group). Correlations at 90 μs (dark gray) correspond to distances of ~ 2 -4 Å. At 1 ms (light gray), additional correlations, corresponding to distances of up to ~ 4 -6 Å are observed. B) Intermolecular distances involving H50 in the crystal structure of Posaconazole that yielded correlations in the 2D ^1H - ^1H RFDR correlation spectra (CCDC database identifier YIMVUO, ³⁶). Intermolecular distances are shown by pink dotted lines, while intramolecular distances are shown by black dotted lines.