A curious case of dynamic disorder in pyrrolidine rings elucidated by NMR crystallography

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A pharmaceutical exhibits differing dynamics in crystallographically distinct pyrrolidine rings despite being nearly related by symmetry, with one performing ring inversions while the other is constrained to torsional librations. Using 13C solid-state magic-angle spinning (MAS) NMR and DFT calculations, we show that this contrast originates from C–H···π close contacts and less efficient C–H···π intermolecular interactions observed in the transition state of the constrained pyrrolidine ring, highlighting the influence of the crystallographic environment on the molecular motion.

Pharmaceutical products are most often manufactured in their solid forms, benefitting the patient with a convenient route of administration. During the development stage, this solid form is thoroughly characterized in order to identify potential risks associated with stability, polymorphic conversion, and the ability to form hydrates or solvates. Characterization may include X-ray crystallography and using the derived structural model to assess the risks of making a particular solid form into a medicine. The occurrence of crystallographic disorder arising from mobility (i.e. dynamic structural disorder) or the accessibility of multiple conformations/orientations (i.e. static structural disorder) poses several challenges in the risk assessment, due in part to the uncertainty on the atomic positions.

Solid-state NMR spectroscopy is a powerful tool for investigating crystallographic disorder, with the potential to exploit several pharmaceutically-relevant nuclei (1H, 13C, 15N) and the ability to probe specific sites in the structure. Further, NMR crystallography is capable of distinguishing static from dynamic structural disorder, has a history of investigating dynamics in pharmaceuticals, and can be used to improve structural models. Conversely, the presence of dynamics may not be immediately apparent from X-ray data, especially for data acquired at low temperatures due to a “freezing” of the motion.

Here, we combine solid-state NMR and DFT calculations in an NMR crystallography approach to investigate a development risk of making a pair of crystals, with a GIPAW calculated shift shown below as sticks. The compound investigated herein, 1a, consists of the salt (the counterion is referred to as “a”) of a pharmaceutical compound (1) in a 1:1 stoichiometric equivalence. The structural model, determined by X-ray crystallography at 150 K, suggests the presence of a pair of 1a related by C2 pseudosymmetry, with the disorder in one of the pyrrolidine groups of 1 breaking this symmetry. As shown in Fig. 1a, where the red dotted lines represent the rest of the undisclosed structure, a pyrrolidine group appears to be relatively “ordered” (henceforth referred to as Cdis), while the other group appears to be disordered (henceforth referred to as Cord) over two positions with occupancies of 0.5 each. However, while Cdis appears to be ordered, the situation is ambiguous as its anisotropic displacement ellipsoids have some distortions (see Figure S3 of the ESI), suggesting the presence of vibrations. The crystallographic environment surrounding the two pyrrolidine groups differ in that Cdis interacts more closely with the counterion a while Cord interacts primarily with other molecules of 1. All contacts (within 3 Å) involving the pyrrolidine groups are shown in Figure S4 of the ESI. In order to confirm the contrast in the dynamics of the pyrrolidine groups, variable temperature 1H-13C cross polarisation (CP) magic-
angle spinning (MAS) solid-state NMR experiments and \(^{13}\text{C}\) spin-lattice relaxation time measurements, \(T_1(\text{C})\), have been performed.

When there are two molecules in the asymmetric unit, a doubling of \(^{13}\text{C}\) resonances can be observed if the crystallographic environments between otherwise chemically equivalent sites are sufficiently distinct. As shown in Fig. 1b, a \(^{13}\text{C}\) chemical shift difference of 2.2 ppm is observed between \(\text{C}^{\text{ord}}\) (δ\(^{13}\text{C}\) = 26.7 ppm) and \(\text{C}^{\text{dis}}\) (δ\(^{13}\text{C}\) = 24.5 ppm). The \(^{13}\text{C}\) signals have been assigned to their sites in the structural model using gauge-including projector augmented-wave (GIPAW)\(^{30}\) DFT calculations as part of CASTEP.\(^{31}\) The calculations were performed for both conformations of \(\text{C}^{\text{dis}}\), and the average GIPAW calculated δ\(^{13}\text{C}\) chemical shifts are 26.7 ppm and 24.3 ppm for \(\text{C}^{\text{ord}}\) and \(\text{C}^{\text{dis}}\), respectively, resulting in a computed difference of 2.4 ppm. These calculated results, shown on Fig. 1b as sticks, are in excellent agreement with the experimental results.

As the pyrrolidine groups consist of saturated heterocycles, they can exhibit dynamics in the form of ring inversions,\(^{32,33}\) analogous to those observed in cyclohexane.\(^{30,34}\) In order to investigate the dynamics, \(^{1}\text{H}-^{13}\text{C}\) solid-state CP MAS NMR and \(T_1(\text{C})\) measurements were performed at 10°C steps between 0°C and 60°C. As shown in Fig. 2a, there are no significant changes to the \(^{13}\text{C}\) chemical shifts of \(\text{C}^{\text{ord}}\) or \(\text{C}^{\text{dis}}\) as the temperature is increased, and this is also true for all the other resonances (not shown on the figure). Supported by differential scanning calorimetry (see Figure S5 of the ESI), this suggests that no phase changes or major structural changes are occurring between these temperatures.

The \(T_1(\text{C})\) at 20°C for \(\text{C}^{\text{ord}}\) and \(\text{C}^{\text{dis}}\) were 9.7 s and 1.4 s, respectively, and all \(T_1(\text{C})\) values are shown on Fig. 2b and have been tabulated in Table S1 of the ESI. These short \(T_1(\text{C})\) suggest that both \(\text{C}^{\text{ord}}\) and \(\text{C}^{\text{dis}}\) are dynamic. To place these values into context, the \(T_1(\text{C})\) of the rigid carbons of 1a are >100 s, whereas the \(T_1(\text{C})\) of rotating methyl groups on 1a are 11 s and 15 s at 20°C. The relationship between \(T_1(\text{C})\), the correlation times (\(\tau_\text{c}\)), and the activation energy are well known, and have been interpreted using the Bloemergen-Purcell-Pound model.\(^{35-37}\) Assuming it follows the Arhenuis equation, measuring the \(T_1(\text{C})\) relaxation times as a function of the temperature allows for the activation energy to be extracted (see Eq. S1 to S3 of the ESI). As we have employed a fixed dipolar coupling constant using a C-H bond length of 1.09 Å and assume no motional averaging of the dipolar coupling, the value of \(\tau_\text{c}\) is an estimate and was not further considered in our analysis.

A plot of \(T_1(\text{C})\) as a function of the temperature is shown in Fig. 2b, with the fits using Eq. S1 to S3 showing in excellent agreement with the experimental results (\(R^2 > 0.96\)). The extracted activation energies are 11 ± 2 kJ mol\(^{-1}\) for \(\text{C}^{\text{ord}}\) and 16 ± 3 kJ mol\(^{-1}\) for \(\text{C}^{\text{dis}}\), with all parameters being summarized in Table 1. The higher activation energy of \(\text{C}^{\text{dis}}\) has been attributed to dynamics in the form of ring inversions, which is also supported by the X-ray structure. In contrast, having a single favourable conformation, short \(T_1(\text{C})\) relaxation times, and anisotropic displacement ellipsoids suggesting the presence of vibrations, we associate the lower experimental activation energy of \(\text{C}^{\text{ord}}\) to torsional librations rather than ring inversions. The activation energy of \(\text{C}^{\text{ord}}\) is very similar in value with the calculated energy of 17.2 kJ mol\(^{-1}\) for the pyrrolidine group in proline performing a ring inversion.\(^{38}\)

In order to understand why \(\text{C}^{\text{ord}}\) is capable of exhibiting ring inversions while \(\text{C}^{\text{dis}}\) is only librating, transition state calculations were performed using CASTEP. These calculations search for the energy maximum between two conformations (puckered up & down) using the linear synchronous transit method.\(^{39,40}\) Each model was optimized with constrained unit cell parameters prior to the calculations, and the transition state calculations were performed individually for both \(\text{C}^{\text{ord}}\) and \(\text{C}^{\text{dis}}\). In the case of \(\text{C}^{\text{ord}}\), while only a single position was observed experimentally, a tentative structure for the second conformation was generated through modelling and DFT optimizations.

The calculations performed on the full structures, shown as teal diamonds in Fig. 3, indicate that the two conformations of the pyrrolidine group, puckered up (left) and down (right), have approximately equal energies for \(\text{C}^{\text{dis}}\), whereas a 22.3 kJ mol\(^{-1}\) energy difference is observed for \(\text{C}^{\text{ord}}\). These results suggest that both conformations of \(\text{C}^{\text{dis}}\) are energetically favourable, while only the conformation that was experimentally observed in the structural model for \(\text{C}^{\text{ord}}\) is favourable. Further, the calculated transition state energy barrier for \(\text{C}^{\text{dis}}\) relative to the puckered down conformation is 17.8 kJ mol\(^{-1}\), in excellent agreement with the experimentally measured activation energy of 16 ± 3 kJ mol\(^{-1}\). We propose that this energy barrier is low enough to permit the pyrrolidine ring to undergo dynamics in the form of ring inversions, with both conformations of \(\text{C}^{\text{dis}}\) being accessible. In contrast, the transition state energy of a ring inversion for \(\text{C}^{\text{ord}}\) is 31.7 kJ mol\(^{-1}\) relative to the starting geometry, which is not in agreement with the experimental

Table 1. Experimental thermodynamic parameters obtained from fitting the \(T_1(\text{C})\) in Fig. 2b for 1a using Eq S1 to S3 (see the ESI), including the correlation coefficient \((R^2)\), as compared to the DFT-calculated activation energies performed on complete ring inversions.

| Group | \(E_a\) (kJ mol\(^{-1}\)) | \(\tau_\text{c}\) (s) \(\times 10^{15}\) | \(a\) | \(R^2\) | Comment |
|-------|---------------------|------------------|-----|-------|---------|
| \(\text{C}^{\text{ord}}\) | 11 ± 2 | 4 ± 3 | 0.11 | 0.96 | Experimental |
| \(31.7^\circ\) | 16 ± 3 | 4 ± 2 | 0.10 | 0.98 | Calculated |

\(^{a}\)Calculated using DFT as part of CASTEP (see Fig. 3).
NMR results of 11 ± 2 kJ mol⁻¹. The clear discrepancy between the computational and experimental results for Cord suggests that for this ring, the barrier for a ring inversion is too high, and thus the libration is observed experimentally. To understand why Cdis is exhibiting ring inversions while Cord is only librating, a series of structural models were created. The first set of models consisted of structure 1a but the counterions "a" have been removed while maintaining periodicity (red squares), and the second set consisted of completely isolating either molecule of 1 in a cell enlarged by 9 Å along the a, b, and c axes of the unit cell (purple circles). These models allow interactions arising from the crystal packing to be removed selectively.  

As shown in Fig. 3, there is a clear contribution to the transition state energies from crystal packing. The intermolecular interactions involving Cdis and Cord can be identified based on the molecules involved, either between two molecules of 1 (denoted here as 1−1) or between molecules of 1 and a (denoted here as 1−a). In the case of Cdis, removing the counterion a has lowered the calculated energy barrier by 5.1 kJ mol⁻¹, whereas a reduction of 2.8 kJ mol⁻¹ was observed upon isolating the molecule 1 that has Cord. In contrast, isolating the molecule 1 that has Cord reduced the energy barrier of Cord by 17.2 kJ mol⁻¹, whereas removing the counterion a merely reduced the barrier by 1.2 kJ mol⁻¹. Notably, in the isolated molecule 1, the energy of both conformations of Cord are now nearly the same, and the energy barriers are similar for both Cdis and Cord due to the removal of the intermolecular interactions. Evidently, the intermolecular interactions involving Cdis and Cord are distinct, with 1−1 interactions playing a larger role in the energy barrier for Cord and 1−a interactions being more important for Cdis. 

The interactions in the structural model were analysed in detail and are shown in Fig. 4, illustrating all atoms within distances shorter than the sum of their van der Waals radius and near hydrogen atoms for both conformations (puckered up & down) of both pyrrolidine rings. Cord exhibits mostly 1−1 interactions (Fig 4. a, b), whereas Cdis presents both 1−1 and 1−a interactions (Fig 4. c, d). In the case of Cdis, there are six and eight atoms within this specified radius of any hydrogen atom in the ring when puckered up and down, respectively, compared to eight and ten atoms for Cord in the same conformations. 

The significance of these interactions was further investigated using DFT calculations performed on molecular cluster models (tabulated in Table S2 of the ESI), noting that 1−a interactions have highly stabilizing energies due to their opposing charges. Interestingly, the energies of the 1−a interactions involving Cdis are very similar for both conformations, with values of −166.3 kJ mol⁻¹ and −167.2 kJ mol⁻¹ when puckered up and down, respectively. This difference may have been reflected in the CASTEP calculations as a slightly higher stability of the puckered down conformation of Cdis (see Fig. 3). Meanwhile, there is a much larger disparity in the energies of the interactions involving both conformations for Cord. For example, the contributions from 1−1 interactions from beneath the ring (relative to Fig. 1a) is 51.8 kJ mol⁻¹ when Cord is puckered up (experimentally observed conformation), and 62.3 kJ mol⁻¹ when puckered down (not experimentally observed). The differences in these interaction energies may partially explain the origin of the energy gap of 22.3 kJ mol⁻¹ between both conformations of Cord in the CASTEP calculations performed on the full structure of 1a (see Fig. 3), and why a single librating conformation is observed in the structural model. 

In order to decompose the intermolecular contributions to the energy barriers for the dynamics of Cdis and Cord, the approach discussed above was applied to the transition states obtained from the DFT calculations (see Table S2 of the ESI). In this case, the energies involving specific intermolecular interactions were computed for the transition states and compared to the puckered up / down conformation, for both Cdis and Cord. The energy barrier for the ring inversions of Cdis appears to originate primarily from the weakening of C-H···O interactions originating from 1−a, with a difference of 7.4 kJ mol⁻¹ between the puckered up conformation and the transition state, thus destabilizing the transition state of Cdis. This further supports the results obtained from the calculations presented in Fig. 3b, where removing the counterion reduced the energy barrier of Cdis. However, the overall calculated energy barrier (17.8 kJ mol⁻¹) is still small enough to allow ring inversions to occur. In terms of Cord, the destabilizing 1−1 interactions originate mainly from a build-up of close contacts between neighbouring pyrrolidine hydrogens (C-H···π) and in part due to less efficient C-H···π interactions.
interactions in the transition state. Overall, this imposes a much greater energy barrier for a ring inversion to occur (31.7 kJ mol⁻¹), and results in a higher relative energy for the puckered down conformation. This explains why a significant reduction in the energy barrier for C₅OH was observed in the isolated molecule (cf. Fig. 3a).

In order to investigate the wider significance of the phenomenon investigated here, we have searched the Cambridge Structural Database (version 5.41) for disordered pyrrolidine rings using the structure on Fig 1a as the search query. Full details on the analysis can be found in section 4 of the ESI. We have identified 179 examples where the pyrrolidine ring exhibits structural disorder, with 20 structures having a Z = 2 and a case of contrasting disorder akin to our compound 1a (see Table S3 of the ESI). Further, pyrrolidine ring inversions are shown to have implications on the structure of proline38, 46, 47 and proline-containing peptides.48, 49 Evidently, disorder in pyrrolidine rings is not a rare occurrence, and is likely also the case for other five-membered rings. The approach demonstrated here of combining solid-state NMR and DFT calculations may help to unravel these cases of disorder, while providing a theoretical framework for their origins. Interestingly, while intermolecular interactions have previously been shown to play a role in dynamics,50-54 their influence has been manifested here as two pseudosymmetric pyrrolidine groups exhibiting distinct dynamics.

Conclusions

In conclusion, the disorder observed in C₅H of compound 1a has been attributed to the occurrence of dynamics in the form of ring inversions with an activation energy of 16 ± 3 kJ mol⁻¹. Despite the pseudosymmetry of the structure (Z = 2), ring inversions were only observed for C₅H while C₆H was constrained to torsional librations with an activation energy of 11 ± 2 kJ mol⁻¹. DFT calculations suggest that the constraints on C₆H originate from neighbouring C–H:C–H and less effective C–H:νC–H intermolecular interactions between 1–1 in the transition state and the ring inversion product. Meanwhile, the counterion plays a more direct role in the ring inversions of C₆H, albeit with a weaker effect. The strategy of combining solid-state NMR and DFT calculation has allowed thorough details on the disorder to be extracted individually for both rings, overall providing significant improvements to the structural understandings of 1a.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

† The expected mean absolute error in absolute interaction energies (IE) for PBE-TS calculations is 1.5 kJ mol⁻¹,55 and errors in relative IEs are expected to be even smaller.

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Summary. Pseudosymmetric pyrrolidine groups exhibiting distinct dynamics are investigated by solid-state NMR and DFT, uncovering the origins to this contrast.