The Rich Solid-State Phase Behavior of L-Phenylalanine: Disappearing Polymorphs and High Temperature Forms

Herma M. Cuppen,† Mireille M. H. Smets,‡ Annika M. Krieger,‡ Joost A. van den Ende,‡ Hugo Meekes,‡ Ernst R. H. van Eck,† and Carl Henrik Görbitz†

†Radboud University Nijmegen, Institute for Molecules and Materials, Heyendaalseweg 135, 6525 AJ Nijmegen, The Netherlands
‡Department of Chemistry, University of Oslo, N-0315 Oslo, Norway

ABSTRACT: After years of controversy over the solid state structure of the essential amino acid L-phenylalanine, four different polymorphic forms were published recently. The common form I has symmetry P2₁, with four molecules in the asymmetric unit (Z' = 4), similar to form III, but with a different arrangement of molecular bilayers. Form II, obtained from the hydrate at very low humidity, is unrelated to forms I and III, as is the high-density form IV. The present investigation demonstrates that this prototype aromatic amino acid has two additional high-temperature phases Ih and IIIh obtained from form I and form III above 458 and 440 K, respectively, when flipping between two alternative side-chain conformations becomes dynamic and causes pairs of molecules, initially crystallographically independent, to become equivalent above a sharp transition temperature. These abrupt and reversible phase changes occur with a reduction of Z' from 4 (low T) to 2 (high T) and modified crystal symmetry. We furthermore experienced an example of disappearing polymorph for form I which after growing form III in one of our laboratories could no longer be crystallized at room temperature. In contrast, form III crystals may be irreversibly converted to form I crystals as a result of sliding of molecular bilayers in the crystal at elevated temperature. No conversions between the high-temperature forms Ih and IIIh were found. The remarkable crystallographic results are here corroborated by Molecular Dynamics and metadynamics simulations of the form I – form III system.

1. INTRODUCTION

Polymorphism is the ability of a compound to crystallize in multiple crystal structures. In general, polymorphic forms have different properties, such as dissolution rate, and for this reason formal approval of pharmaceuticals is often linked to a specific polymorphic form. Stability of this form is hence crucial, and phase transitions inside the solid are undesirable. However, the understanding of solid-to-solid polymorphic transitions in molecular crystals is still in its infancy. Here we present a combined computational and experimental study of the solid-to-solid transitions between polymorphic forms of L-phenylalanine. Phenylalanine is often considered as a prototypical example of a large class of important aromatic compounds. Understanding its phase transition behavior is therefore a first step toward polymorph control. Crystal structure prediction has shown that the structural landscape of L-phenylalanine is very rich and it has been speculated that all solid forms may not have been characterized yet. Here we will show that this is indeed the case by presenting two new high T forms.

Amino acids were among the first chiral molecules to be investigated by X-ray crystallographic methods, but for anhydrous L-phenylalanine (L-Phe), L-asparagine, L-tryptophan, L-lysine, and L-arginine solid-state structures have been published only in recent years, for the latter two derived from powder X-ray diffraction data. The first single crystal investigation of the common form I of phenylalanine (F-I), as α-Phe, was reported in 1990 [Cambridge Structural Database (CSD) refcode SIMPEF†], but low resolution, a high final R-factor, and some unexpectedly short intermolecular H···H distances led to continued speculations concerning the correctness of the C2 space group assignment with two molecules in the asymmetric unit (Z' = 2). In 2013, Williams et al. used powder X-ray diffraction to elucidate the structure of a second polymorph, form II (F-II, QQQAUJ04), “stable only under rigorously dry conditions”, which can be reversibly converted to a hemihydrate and monohydrate upon increased relative humidity. Mossou et al. further reported a new monoclinic structure (P2₁, Z' = 4, QQQAUJ03), which we have called form III (F-III). Single crystals were grown by a rather elaborate procedure from a neutral aqueous solution containing 10 mg mL⁻¹ L-Phe, 15% of both polyethylene glycol and propan-2-ol, and 0.05 M NaCl.

In parallel with the work by Mossou et al., we discovered a fourth polymorph (F-IV, QQQAUJ07), but more importantly that the quality of F-I crystals could be significantly improved by using, rather than pure water, solutions containing 5–10%...
formic or acetic acid in simple vapor diffusion experiments. With excellent crystals at hand, we were subsequently able to establish that the space group of F-I, in contrast to previous findings, is P2₁ with Z′ = 4 (QQQUJ05).

Here, we will focus on forms I and III which have, as mentioned earlier, very similar packing motifs. The cell parameters of both forms are given in Table 1. The monoclinic axis changes from form I to form III, but the volumes as well as the axes are very similar. Both polymorphic forms contain roughly two different conformers: differing in the dihedral connecting the phenyl ring. In both cases the structures consist of bilayers with alternating conformers. The difference between two structures lies in the positioning of these layers with respect to each other. A shift of every other bilayer along the a axis results in a transition between the two forms. Mere torsional rotations of the phenyl group cannot transform form I into form III.

A central question, given the close relationship between the F-I and F-III structures, is whether low-energy paths exist for converting one form into the other. Here we will present a combination of experimental and computational results to show that low-energy paths indeed exist. However, there are convincing feature around 440 K. This feature is visible both in the thermal vibrations are obviously high at such an elevated temperature (Figure 2a), but other changes compared to the corresponding structures obtained at 293 (QQQUJ06) and 105 K are not apparent. There is, however, a gradual change in the C2—C3—C4—C5 torsion angles for the three temperatures that tends to make the difference between neighboring molecules A and B smaller, as is also the case for C and D (Table 2). The same trend can be observed upon heating powder samples of form I in SS-NMR (see Figure 3). The features in the left panel are due to the C atoms in the phenyl group; the middle features due to C2-atoms and the right panel due to C3-atoms. At low temperatures, a Z′ = 4 signature can be observed, especially for C2 and C3 with a clear separation between A and B, and between C and D. The asymmetric unit of the new polymorph, which we have called F-Ih, has only two molecules and is shown in Figure 2b. The somewhat unphysical appearance of the thermal displacement ellipsoids in the aromatic ring suggests that the refined conformation does not represent an energy minimum, but rather results from rapid flipping between two side chain orientations more or less identical to those observed at 423 K. This is manifested, e.g., by short intermolecular distances, such as 2.02 Å for H61A—H91A (x, y + 1, z), as noted by Weissbuch et al. in their original contribution. Indeed, by omitting reflections h + k = 2n + 1 from the 423 K data sets very similar ellipsoids are obtained, Figure 2c, and when

<table>
<thead>
<tr>
<th>Form</th>
<th>l</th>
<th>l′</th>
<th>I</th>
<th>Ih</th>
<th>III</th>
<th>III</th>
<th>IIIh</th>
<th>IIIh</th>
</tr>
</thead>
<tbody>
<tr>
<td>space group</td>
<td>P2₁</td>
<td>P2₁</td>
<td>P2₁</td>
<td>C2</td>
<td>P2₁</td>
<td>P2₁</td>
<td>P2₁</td>
<td>B₁</td>
</tr>
<tr>
<td>c (Å)</td>
<td>31.0175</td>
<td>31.5233</td>
<td>31.879</td>
<td>32.01</td>
<td>8.7980</td>
<td>8.7953</td>
<td>8.7956</td>
<td>5.3561</td>
</tr>
<tr>
<td>β (deg)</td>
<td>96.9220</td>
<td>96.6441</td>
<td>96.448</td>
<td>96.14</td>
<td>90.120</td>
<td>90.041</td>
<td>90.045</td>
<td>110.604</td>
</tr>
<tr>
<td>V (Å³)</td>
<td>1622.12</td>
<td>1662.40</td>
<td>1694.8</td>
<td>1704</td>
<td>1662.24</td>
<td>1662.96</td>
<td>1671.6</td>
<td>1707.6</td>
</tr>
<tr>
<td>Z/Z′</td>
<td>8/4</td>
<td>8/4</td>
<td>8/4</td>
<td>8/2</td>
<td>8/4</td>
<td>8/4</td>
<td>8/4</td>
<td>4/2</td>
</tr>
<tr>
<td>T (K)</td>
<td>105</td>
<td>293</td>
<td>424</td>
<td>463</td>
<td>100</td>
<td>293</td>
<td>343</td>
<td>443</td>
</tr>
</tbody>
</table>

*Previous work is in italics. Refcode QQQUJ05, ref 9. Refcode QQQUJ06, ref 9. Refcode QQQUJ03, ref 8. Alternative space group setting with twice the volume.*
conformations from the lower temperature $P_2_1$ structure are used as a starting point for structure refinement, it is possible also to fit a model incorporating disorder. Neither the $R$-factor nor the thermal ellipsoids are significantly improved by this measure, but two distinct conformations become easily visible (Figure 2d). The reduction from 4 to 2 for $Z'$ is associated with a change of space group from $P_2_1$ to $C_2$, meaning that the original space group assignment of Weissbuch et al. is in fact correct above 458 K. The illustrations of the unit cell at 423 and 463 K in Figure 4a, b confirm that there are no major changes with respect to the overall crystal packing arrangement.

The collection of diffraction data for l-Phe at 463 K was not an entirely straightforward process, as mounted specimens invariably appeared to drop off after 30 min or less. It was initially believed that the hydrophobic surface of the crystal made it hard for any type of glue to stick to it, but when further data collection attempts, with the selected specimen totally embedded in epoxy glue inside a borosilicate capillary, also failed (the crystal seemed more or less to explode, leaving just an imprint of its previous location), it was realized that the problems resided with the stability of the crystal. According to the literature, l-Phe decomposes at 483 K, but we carefully monitored crystals lying on a glass slide at 470 K and found rapid evaporation even at this temperature. A complete data set of reasonable quality was eventually collected in about 25 min, using a big crystal that permitted short exposure times of 2 s.

Due to the $\beta$ angle being close to $90^\circ$, all F-III crystals tested showed twinning (monoclinic emulating orthorhombic, SHELX command TWIN 1 0 0 0 0 1 0 0 0 0 1). The fraction

<table>
<thead>
<tr>
<th>$T$ [K]</th>
<th>105 K</th>
<th>293 K</th>
<th>423 K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecule A</td>
<td>95.0</td>
<td>92.6</td>
<td>87.4</td>
</tr>
<tr>
<td>Molecule B</td>
<td>61.1</td>
<td>60.6</td>
<td>62.2</td>
</tr>
<tr>
<td>Molecule C</td>
<td>59.1</td>
<td>59.3</td>
<td>60.9</td>
</tr>
<tr>
<td>Molecule D</td>
<td>97.0</td>
<td>95.1</td>
<td>90.5</td>
</tr>
</tbody>
</table>
of the minor component was typically in the range 0.05−0.15 with SHELX BASF = 0.097 for the 343 K data set in Table 1. Downloading and testing the previously published structure of F-III (CSD refcode QQQAUJ038) yielded a lower value of 0.052 for the minor component, for a reduction of the R-factor from 0.0641 to 0.0533.

The observations during transition from F-III to F-IIIh upon heating very much mirror those for the F-I to F-Ih transition, albeit at a slightly lower temperature of about 440 K, as indicated initially by DSC. The asymmetric units at 343 and 443 K are shown in Figure 2e,f. Dynamic disorder above the transition temperature in this case leads to a new P2₁ unit cell with Z′ = 2 and Z = 4 (Table 1 and Figure 4c,d). Table 1 also lists the alternative B2₁ space group setting shown in Figure 5, which has more or less the same unit cell parameters as the lower temperature P2₁ structure and retains the Z-value of 8.

3. MOLECULAR DYNAMICS SIMULATIONS OF HIGH T FORMS

To confirm that the increase in symmetry from the low temperature F-I and F-III forms to the high temperature F-Ih and F-IIIh forms, respectively, are indeed due to fast rotation of phenyl groups, we performed molecular dynamics simulations. Simulations are performed starting from either form I or form III at room temperature followed by several heating and cooling steps. Cell lengths, cell angles, and C2−C3−C4−C5 dihedral angles for all molecules are recorded during the simulation. Their progression can be observed in Figure 6 for form III. The black lines indicate the dihedral average angle; the colored lines represent the dihedral angles of a selection of individual molecules. The system is initially kept at room temperature for 0.2 ns, the structure is subsequently heated to 430 K and in the next step to 470 K (see vertical dotted lines). The crystal is then cooled again to room temperature. At 400 K, the average values of the dihedral angles of molecules A and D become smaller and of molecules B and C become larger. This is in agreement with the experimental findings (see Table 2). For the “red” molecule C this is because the molecule quickly changes between 55° and 80°. For molecules A and C, the overall dihedral angle reduces. Upon further increasing the temperature to 470 K, all molecules start to rapidly change between both orientations (60° and 90°), consistent with F-IIIh. Cooling leads to F-III.
again, although some local disorder can become frozen in (purple line in Dih B). Similar results are obtained for F-I $\rightarrow$ F-Ih $\rightarrow$ F-I. The high T form has higher symmetry and transforming to the low T form occurs through symmetry breaking. This can be done in two ways and both realizations are fully equivalent. We have repeated the heating and cooling simulations multiple times for both F-I and F-III and recovered the original form in both realizations or mixed forms. This indicates that indeed the conversion to the high T form was complete, without memory of the initial starting structure.

4. DISAPPEARING POLYMORPHS

An added complication of the crystallographic work on F-I is that before a complete data set of adequate quality had been collected, we started to grow F-III crystals using the methodology described by Mossou et al. Once successful (see below), we found that we could no longer obtain F-I crystals; any setup, including crystallizations from plain aqueous solutions (with acetonitrile as the precipitating agent), yielded F-III crystals of decent to nice quality. Even experiments carried out by helpful master students in other laboratories in the Chemistry building of the University of Oslo, which none of the authors had ever accessed, resulted in F-III crystals. Obviously, formation of F-III crystals is kinetically preferred, and we experienced an example of a “disappearing polymorph” as described by Duntiz and Bernstein. Calculations of the energy of optimized geometries of both forms (see next section) suggest that F-III is also the thermodynamically more favorable form with an energy difference of 0.51 kJ/mol molecules. This difference is below the accuracy of the force field applied.

The final experiments with F-Ih were completed with crystals grown in our laboratory in The Netherlands, which is not yet “infected” by F-III microseeds. Interestingly, attempts to grow D-Phe crystals in Oslo without exception yielded F-I crystals, so one enantiomer does not infect the other. It furthermore appears that once high quality crystals have been obtained, either of form I or III, special measures (i.e., use of acetic acid or formic acid as described previously) is no longer needed.

5. RELATIONS BETWEEN THE DIFFERENT FORMS AND POSSIBLE TRANSITIONS

We already mentioned the differences between F-I and F-III in terms of different stacking of layers. This is further detailed in Figure 7. Most layered crystal structures, including, in particular, those of other hydrophobic amino acids, have symmetry operators at bilayer interfaces, which means that the way H atoms (“knobs”) fit into depressions of the opposing surface (“holes”) is the same at both sides of the interface. The unusual lack of this type of symmetry in F-I and F-III complicates this simple construction principle. As seen in Figure 7b, there is a pseudohexagonal array of “holes” where H atoms could fit, but at any instance only half of these can be utilized, either the white ones or the black ones. Moreover, if H atoms from one aromatic monolayer (1) fit into black holes on the opposing surface (2), the H atoms of 2 will automatically fit into white holes on 1. There are no black–black or white–white fits, as either would most probably lead to additional crystallographic symmetry. This means that one can define a direction at each interface, arbitrarily chosen to point in the direction of the black holes, as shown by the yellow arrow for F-III in Figure 7a (l $\rightarrow$ l; l representing the hydrogen bonding groups). This leads to an intriguing relationship between the two polymorphs in terms of stacking directions; the arrows in Figure 4a show that F-I is a $\parallel$ $\leftrightarrow$ $\parallel$ $\rightarrow$ $\parallel$ $\rightarrow$ $\parallel$ sequence, while F-III in Figure 4c is a $\parallel$ $\rightarrow$ $\parallel$ $\rightarrow$ $\parallel$ $\rightarrow$ $\parallel$ sequence. Notably, these cannot be interconverted by conformational changes alone. There is no alternative, third $\parallel$ $\leftrightarrow$ $\parallel$ $\leftrightarrow$ $\parallel$ $\leftrightarrow$ $\parallel$ sequence, as this would simply imply a $180^{\circ}$ rotation of F-III.

Do the higher symmetries of the high temperature polymorphs I and IIIh change this picture? In fact they do not; Figure 4b,d shows that the stacking sequences remain the
smaller and of molecules B and C become larger. Upon cooling the average value of the dihedral angles of molecules A and D become which the temperature changes in the simulations. At 400 K, the vertical dotted lines represent the times at

Figure 6. Cell lengths, cell angles, and C2−C3−C4−C5 dihedral angles (Dih) for a selection of molecules A, B, C, and D during a heating and cooling simulation of form III. The dashed curves indicate the experimental cell parameters of F-III and F-IIIh at 343 and 443, respectively. The vertical dotted lines represent the times at which the temperature changes in the simulations. At 400 K, the average value of the dihedral angles of molecules A and D become smaller and of molecules B and C become larger. Upon cooling the original values are restored.

same. F-Ih and F-IIIh are consequently related to each other in exactly the same way as F-I and F-III.

We have extensively searched for phase transitions between F-I and F-III crystals between ambient temperature and 105/100 K. Differential scanning calorimetric (DSC) analysis starting from F-I, shown in Figure 1, did not show any features that could indicate a phase transition at low temperatures. Because of the similarity between forms I and III, phase transitions can however remain unnoticed in the DSC, where the small differences in enthalpy are spread out over several degrees resulting in smooth DSC traces. Also, the solid state NMR of F-I did not reveal any significant change in the peak profiles or the appearance of new peaks. The calculated spectra of forms I and III (on top of Figure 3) do not show a clear area in chemical shift where a change is expected. SCXRD is more conclusive in that respect. We first tried heating F-Ih and F-IIIh, all the way up to the point where they evaporated, but found no indications of transitions between the two forms. Cooling of F-III crystals led, however, to some interesting results. Out of ten crystals tested, seven kept their overall structure all the way down to ambient temperature, while three abruptly changed to F-I at approximately 393 K. Clearly, there is crystal-to-crystal variation, but we have here confirmed that F-III crystals may sometimes be converted to F-I crystals. It appears that this transition is irreversible, as no examples have been found where F-I crystals are converted to F-III.

6. SIMULATIONS OF F-III TO F-I TRANSITION

Since the experimental time scales of the F-III to F-I transition are rather long, chances that we can observe this transition in MD simulations spontaneously are slim. Indeed, we did not observe this behavior during our standard simulations. For this reason, we apply a metadynamics simulation12,13 where we can “force” the system to go over a barrier. In a metadynamics simulation, a repulsive bias potential is gradually added during the course of the simulation. The bias potential takes the form of Gaussians in collective variable (CV) space and each Gaussian is added at the position in CV space where the system resides at that moment, filling up the free energy well. For our l-phenylalanine simulations, two collective variables (CVs) have been devised to describe the relative movement of two neighboring bilayers along the a (8 Å axis) and b axis (6 Å axis) of the simulation cell. The collection of C2 atoms (= C_n Figure 2) is used as reference to pinpoint the location of the bilayers with respect to each other. The C2 atoms participate in the hydrogen bonded “backbone” in the center of the bilayer and it will therefore have little freedom to move with respect to the rest of the bilayer. By choosing these CVs, the layers are free to move along a and b and also breath along c. We will start our simulations from form I and apply the CVs on the first bilayer (toward form III).

The resulting free energy surfaces are plotted in Figure 8. At 250 K, two free energy minima can be observed. Overlaying the corresponding structures onto the experimentally determined crystal structures (refcodes QQQAUJ059 and QQQAUJ039) reveals that the top free-energy well with positive values of CV 1 corresponds to form I and the bottom well to form III. A local transition between the two forms has therefore indeed occurred with a barrier of 35 meV (dashed line to guide the eye). We want to emphasize that during the relative shift of the bilayers the conformations of the molecules remained intact such that indeed now different conformer pairs are facing each other. Initially, we start with a simulation cell of form I; after the transition half the simulation cell has form III and half form I, since we only steer on one of the four bilayer. A full transition would require shifts of two bilayers. To build up the full free energy surface, the system repeatedly transforms from I to III and back during the simulation. The torsional angles of the phenyl rings are monitored during simulation and these simply fluctuate around an equilibrium position.

During the transitions some cell parameters change. Figure 9 shows the first 120 ps of the simulation (the total simulation lasted 3 ns). Around 25 ps, a transition to form III can be observed; around 90 ps, a transition back to form I occurs. The β angle moves from 98° to 94° and back and the c axis changes slightly. We would like to stress that these are the cell parameters of the simulation cell and not the crystallographic cell parameters. We expect that if the simulation cell completely converts to form III, the β angle would become nearly 90°, in correspondence with the experimental data. The
other cell parameters as well as the total volume remain constant, again in agreement with experimental findings. We did not observe a significant enthalpy change between form I and form III and indeed the difference in free energy between the two minima in Figure 8 is within the uncertainty of the method. Hence, these simulations do not allow us to conclude

**Figure 7.** (a) Same part of the crystal structure of F-III at 343 K as highlighted by a gray shade in Figure 4c, but with inclusion of all H atoms. The side chains of molecules C and D are in space-fill, of A and B in wireframe representation (C2 as a sphere). (b) The C–D surface in F-III. The corresponding C–D surface of F-I as well as the A–B surfaces of both polymorphs are indistinguishable from what is shown here. Depressions (or holes) where H atoms of the opposing aromatic rings would be located are shown as circles; see text for details. (c) Fit of side chain H71A and H71B atoms (polar amino acid heads are omitted) into “black” holes in the C–D surface of F-III. (d) Similar fit into “white” holes in F-I (right). The dashed yellow arrow shows, for the selected H atom in c) (yellow sphere), an almost vertical 3.6 Å long low-energy path along the 8.8 Å axis as the result of bilayer slide during the phase transition.

**Figure 8.** Free energy in meV as a function of the shift of the interface converting form I into form III along a (8 Å axis, CV 1) and b axis (6 Å axis, CV 2) at 250 K. Fractional coordinates are used along the x- and y-axes, the vertical separation between the energy minima of I and III is about 0.45a (or 3.9 Å), in excellent agreement with the 3.6 Å path indicated in Figure 7.

**Figure 9.** Cell parameters and cell volume of the simulation cell during the first part of the metadynamics simulation at 250 K. The evolution of CV 1 is given in the bottom panel to indicate the transition between forms.
anything about the most stable form at this temperature. Both structures are nearly equally stable and the driving force for a transition will therefore be small.

The wells are elongated in the CV 2 direction, which indicates that there is quite some flexibility in movement along the b axis. The minimum free energy path across the saddle point requires some flexibility in this direction during the transition. Our simulations of the transition do not show any expansion between the layers to accommodate the movement, but we believe that the movement along b relaxes the need for an expansion. There is a clear anisotropy in the shifting direction (dashed versus dotted lines in Figure 8), both in barrier (35 vs 65 meV) and in mechanism. The dashed line corresponds to the short distance transition indicated schematically by a yellow arrow in Figure 7, the dotted line with the long distance transition.

A barrier of 35 meV seems very small, and if indeed true, this would mean that the transition between form I and III would readily occur in an experimental setting. The transition, however, involves a cooperative motion and as we have shown earlier for dl-norleucine,\textsuperscript{14} the barrier of this process scales linearly with the number of molecules involved in the movement. The barrier of 35 meV hence corresponds to the free energy barrier involved in sliding a 29 Å by 28 Å area from a F-I to a F-III orientation or the other way around (Figure 10). Moving a 230 nm² area would already lead to a barrier of 1 eV, which is roughly the limit for a thermally activated process around 400 K (1–1.3 eV barriers correspond to a time scale from seconds to hours). Transforming a macroscopic crystal by single-step cooperative motion is therefore out of the question. Fortunately, cooperative motion does not have to proceed simultaneously throughout the whole crystal. Crystal defects like disorder or stacking faults can give rise to grain boundaries where only the molecules between these grain boundaries need to shift. Whether a macroscopic crystal can transform between F-I and F-III depends on the defect density, which explains the large crystal-to-crystal variation that was observed experimentally. In general, the defect density is not very high, since no appreciable diffuse scattering was observed for any crystal tested.

7. DISCUSSION AND CONCLUSIONS

Phase transitions in a related class of materials, racemates or pseudoracemates of aliphatic amino acids, have been studied in detail in the past. Similarly to forms I and III of L-Phe, the molecules in these structures are arranged in bilayers that are interconnected through relatively strong hydrogen bonds. The phase transitions involve relative shifts between these bilayers, in some cases accompanied by a conformational change. We and others have recently reviewed the phase transitions in this class of materials\textsuperscript{14–28} comparing a range of macroscopic quantities such as the enthalpy of transition, volume change, the amount of hysteresis in the transition temperature, and cyclability of the transition, and found distinctly different behavior for the transitions involving only shifts as compared to the phase transitions that involved a large conformational change. Although all phase transitions in aliphatic amino acid crystals are first-order and proceed through nucleation-and-growth, the shifts probably do not occur in a molecule-by-molecule fashion, but cooperatively with many molecules shifting at the same time.\textsuperscript{14,16–28} Conformational changes, on the other hand, are more likely to proceed one molecule at a time.

L-Phe serves as an excellent test system to put these concepts to the test: cooperative shifts of bilayers can result in phase transitions between forms I and III, whereas torsional changes in a molecule-by-molecule fashion will lead to new crystal structures (Ih and Ihh).

Finally, the question remains whether F-I or F-III is the stable structure at room temperature. The fact that F-I showed disappearing polymorph behavior and we were unable to obtain F-I in the Oslo laboratories after crystallization of F-III suggests that F-III is the stable room temperature structure. However, at elevated temperatures F-III was observed to convert in some cases to F-I, indicating the presence of an enantiotropic transition from F-III to F-I somewhere between 300 and 440 K.

8. METHODOLOGY

8.1. Differential Scanning Calorimetry. DSC measurements were taken with a Mettler Toledo DSC1 calorimeter with a high sensitivity sensor (HSS8), in combination with LN2 liquid nitrogen cooling, a sample robot and STAR software 13.00a. Powder samples and single crystals of L-phenylalanine were heated and cooled with 2 K/min in the temperature range of 133 to 473 K. Samples of a few milligrams were sealed in an aluminum pan (40 μL) and the heat flow as a function of temperature was measured with

![Figure 10](image-url)
respect to an empty reference pan. The calorimeter was calibrated with the melting points of indium (T_m = 429.5 K and ∆H_m = −28.13 J/g) and zinc (T_m = 692.85 K and ∆H_m = −104.77 J/g), both supplied by Mettler Toledo.

8.2. Single Crystal X-ray Diffraction. Crystal data, data collection, and structure refinement details are summarized in Table 1. X-ray data were collected on a Bruker D8 Venture single crystal CCD diffractometer with Mo Kα radiation (λ = 0.71073 Å). Data acquisition, integration/reduction, and absorption correction were carried out by the programs APEX2, SAINT, and SADABS, respectively, while SHELXT and SHELXL2014 were used for structure solution and refinement. All O, N, and C atoms were refined with anisotropic displacement parameters. The amino H atoms of F-I at 423 K were refined isotropically, other H atoms were included in calculated positions and treated as riding: N–H = 0.89 Å, C–H = 0.93–0.98 Å with U_eq(H) = 1.5 U_eq(C-methyl/N-amino) and 1.2U_eq(C) for other H atoms. No constraints or restraints were applied. Molecular images and packing graphics were generated using the program Mercury.

8.3. Solid-State Nuclear Magnetic Resonance. Solid-state nuclear magnetic resonance (NMR) spectra of l-Phe were measured on a Varian VNMRS 400 MHz spectrometer, operating at a magnetic field of 9.4 T (Larmor frequencies of 399.94 MHz for 1H and 100.57 MHz for 13C). 13C NMR spectra were measured with a Chemagnetics 3.2 mm APEX probe using 1H → 13C cross-polarization (CP), magic angle spinning (MAS), and SPINAL decoupling for temperatures between 128 and 423 K. The powder spectra of l-Phe mixed with KBr were recorded at an MAS frequency of 10 kHz with radio frequency (RF) field strengths of 50 kHz for 1H and 60 kHz for 13C during cross-polarization and 80 kHz for 1H SPINAL decoupling with a pulse length of 5 μs and a phase of 6°. KBr was used both in powder samples to adjust the magic angle setting at each temperature on the spinning side bands of 79Br. Adamantane was used as reference sample for the chemical shift; the 13C peak with the highest chemical shift value corresponds to the CH2 of adamantane at 38.48 ppm. The spectra were processed using the matNMR processing package that runs under Matlab.

8.4. Molecular Dynamics Simulations. The interactions between and within the molecules were described by the AMBER force field. For the nonbonded interactions, a cutoff of 10 Å and an Ewald summation with a precision factor of 10−6 were used. The molecules were fully flexible throughout the simulations.

Well-tempered (WT) metadynamics simulations were performed using the LAMMPS molecular dynamics simulation package in combination with the COLVARS module, to which several modification have been applied (see below). Input files were generated with the help of VMD to produce a simulation cell consisting of 3 × 5 × 2 crystallographic unit cells of form I with a total of 240 molecules. Simulations were performed in the anisotropic isothermal–isobaric (NPT) ensemble with barostat and thermostat parameters of 0.4 and 0.04 ps, respectively, to simulate at a constant pressure of 1 atm. A time step of 0.5 fs was used. The system was allowed to equilibrate for 10 ps, before the metadynamics was switched on.

The two collective variables (CVs) that have been chosen describe the relative movement of two neighboring bilayers along the a and b axis of the simulation cell, respectively. This was achieved by calculating the distance in fractional coordinates between the α–C coordinates of pairs of molecules in opposing bilayers whose phenyl groups are facing each other. Since each full bilayer consists of 60 molecules, this resulted in 30 pairs. Unwrapped coordinates were used and average distances were taken (see Figure 11). Since the

Figure 11. Depiction of collective variables. The CV are the average distance in fractional coordinates between the α–C coordinates of pairs of molecules (red and green) in opposing bilayers whose phenyl groups are facing each other. CV 1 uses the fractional distance along the a axis, CV 2 along the b axis. A relative shift between two layers along a would result in a transition from F-I to F-III and vice versa. This results in a change in relative position between the red and the green spheres (schematically depicted by the dashed arrow).

ASSOCIATED CONTENT
Accession Codes
CCDC 1876808–1876813 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

AUTHOR INFORMATION
Corresponding Author
*E-mail: h.cuppen@science.ru.nl.

ORCID
Herma M. Cuppen: 0000-0003-4397-0739
Mireille M. H. Smetts: 0000-0003-1938-2099
Hugo Meekes: 0000-0001-9236-2129

Notes
The authors declare no competing financial interest.
ACKNOWLEDGMENTS

The authors are grateful for financial support from the VIDI research program 700.10.427 financed by the Dutch Organisation for Scientific Research (NWO) and the ERC grant from the European Research Council (ERC-2010-StG, Grant Agreement 239510-KISMOL). Support of the Dutch Organisation for Scientific Research (NWO) for the solid-state NMR facility for advanced materials science in Nijmegen is gratefully acknowledged. We thank Gerrit Janssen, Hans Janssen, and Jan Schoonbrood for support with the solid-state NMR measurements, Erik de Ronde and Paul Tinnemans for technical support, and René de Gelder for stimulating discussions.

REFERENCES

(16) Smets, M. Exploring the mechanism of solid-state phase transitions in molecular crystals; Ph.D. thesis; Radboud University, 2018.

Downloaded from acs.cgd.eAPSf14655 on 2019-11-19 15:26:09.


