On the stacking disorder of DL-norleucine

Christian Czech,^a Jürgen Glinnemann,^a Kristoffer E. Johansson,^b Michael Bolte^a and Martin U. Schmidt^{a*}

^aInstitut für Anorganische und Analytische Chemie, Goethe-Universität, Max-von-Laue-Strasse 7, D-60438 Frankfurt am Main, Germany, and ^bInstitut for Farmaci, Københavns Universitet, Universitetsparken 2, DK-2100 København, Denmark. *Correspondence e-mail: m.schmidt@chemie.uni-frankfurt.de

Received 15 May 2017

Accepted 21 August 2017

Edited by F. P. A. Fabbiani, Georg-August-Universität Göttingen, Germany

Keywords: diffuse scattering; disorder; lattice-energy minimizations; dispersion-corrected density functional theory.

CCDC reference: 1570080

Supporting information: this article has supporting information at journals.iucr.org/b

DL-Norleucine (2-aminoheptanoic acid, $C_6H_{13}NO_2$) forms a double-layer structure in all known phases (α , β , γ). The crystal structure of the β -phase was redetermined at 173 K. Diffraction patterns of the α - and β -phases frequently show diffuse streaks parallel to c^* , which indicates a stacking disorder of the layers. A symmetry analysis was carried out to derive possible stacking sequences. Lattice-energy minimizations by force fields and by dispersion-corrected density functional theory (DFT-D) were performed on a set of ordered model structures with $Z = 4, 8$ and 16 with different stacking sequences. The calculated energies depend not only on the arrangement of neighbouring double layers, but also of next-neighbouring double layers. Stacking probabilities were calculated from the DFT-D energies. According to the calculated stacking probabilities large models containing 100 double layers were constructed. Their simulated diffraction patterns show sharp reflections for $h + k = 2n$ and diffuse streaks parallel to c^* through all reflections with $h + k = 2n + 1$. Experimental single-crystal X-ray diffraction revealed that at 173 K norleucine exists in the β -phase with stacking disorder. After reheating to room temperature, the investigated crystal showed a diffraction pattern with strong diffuse scattering parallel to c^* through all reflections with $h + k = 2n + 1$, which is in good agreement with the simulated disordered structure.

1. Introduction

DL-Norleucine [2-aminoheptanoic acid, $C_6H_{13}NO_2$, Fig. 1(a)] is an amino acid but not one of the 20 standard amino acids found in natural proteins. Norleucine is of special interest due to its structural and steric similarities to the natural amino acid methionine (Fig. 1b). For example, one can replace methionine by norleucine in proteins in order to investigate the role of methionine on the protein structure and function (Cirino *et al.*, 2003).

DL-Norleucine crystallizes in three different phases α , β and γ (Mathieson, 1953; Mnyukh *et al.*, 1975; Dalhus & Görbitz, 1996). With increasing temperature, the compound undergoes temperature-dependent reversible phase transitions $\beta \rightleftharpoons \alpha \rightleftharpoons \gamma$. The transition $\beta \rightleftharpoons \alpha$ occurs between about 120 K and 373 K with a large hysteresis. The transition is kinetically

Figure 1
Chemical formula and numbering of (a) L-norleucine and (b) L-methionine.

hindered and the crystal size and quality influence the speed of the phase transition (Smets *et al.*, 2015). In contrast, the polymorphic transition between the α - and the γ -phases, which occurs at 390–391 K, is fast and, in principle, fully reversible without hysteresis (Anwar *et al.*, 2007; Coles *et al.*, 2009; Smets *et al.*, 2015). However, some single crystals got highly damaged after the transition from the γ - to the α -phase upon cooling (Smets *et al.*, 2015). Norleucine is often used as a test system to investigate such phase transitions and thermal behaviour (Tuble *et al.*, 2004; Anwar *et al.*, 2007; Coles *et al.*, 2009; Zahn & Anwar, 2013; van den Ende *et al.*, 2015).

The first investigation of the crystal structure of DL-norleucine (α -phase) was published by Mathieson (1953). The crystals exhibited the space group $P2_1/a$ and cell parameters of $a = 9.84$, $b = 4.74$, $c = 16.56$ Å, $\beta = 104.5^\circ$ at room temperature. The molecules are zwitterionic. Neighbouring molecules are connected by three $N-H \cdots O$ hydrogen bonds between the NH_3^+ and COO^- groups, resulting in a double layer parallel to (001) (see Fig. 2a). Adjacent double layers are connected only by van der Waals interactions between the aliphatic side chains. According to the classification system of Görbitz *et al.* (2009), the hydrogen-bond network corresponds to the type Id–Id/I. This molecular arrangement is very common for racemic amino acids and is also found, for example, in DL-isoleucine (Dalhus & Görbitz, 2000), DL-norvaline (Görbitz, 2011), DL-methionine (Mathieson, 1952) and DL- α -amino-n-butyric acid (Ichikawa & Iitaka, 1968; Görbitz *et al.*, 2012).

Figure 2

Experimental crystal structures of DL-norleucine. (a) α -Norleucine with the stacking sequence L–L (Harding *et al.*, 1995) and (b) β -norleucine with the stacking sequence L–M (this work; data measured at 173 K, major occupied atomic positions only) viewed along the b axis. The unit cells have been transformed to $P2_1/a$ and $I2/a$ for a better comparison of the structures. The letters R and S denote the configuration of the asymmetric carbon atom $C2$.

For the natural amino acid DL-methionine, Mathieson (1952) found two polymorphs, α and β . Both polymorphs have the same hydrogen-bond pattern as DL-norleucine. The α -phases of both compounds exhibit the same molecular packing, identical space groups ($P2_1/a$) and similar lattice parameters. The β -phase of DL-methionine consists of the same double layers as in the α -form, but with a different stacking of the double layers resulting in the space group $I2/a$ with $a' = a$, $b' = b$, $c' = 2c$. In the α -phase, subsequent double layers are generated by a translation of c resulting in the stacking sequence called L–L–L–L (see Fig. 2a). In the β -phase, subsequent double layers are connected by a translation of $a'/2 + b'/2 + c'/2$ resulting in the stacking sequence L–M–L–M (see Fig. 2b). Correspondingly Mathieson (1953) also proposed the existence of a second polymorph (β -phase) for DL-norleucine, which should be isostructural to the β -phase of DL-methionine and have the stacking sequence L–M–L–M, too. Mathieson did not obtain crystals of a pure β -phase. However, he reported that some crystals of DL-norleucine showed diffuse streaks parallel to c^* through the reflections with $h + k = 2n + 1$, pointing to a stacking disorder. Interestingly the intensity distribution in the diffuse streaks varied from crystal to crystal. Because the diffraction patterns contained superstructure reflections with $c^{*'} = \frac{1}{4}c^*$, Mathieson set up different models with $c' = 4c$ and suggested the sequences L–L–M–M or L–L–L–M as predominant in the individual crystals. Although this work was carried out more than 60 years ago, a detailed investigation of the stacking disorder has not been made until today, neither experimentally (by quantitative evaluation of the diffuse scattering) nor theoretically.

Harding *et al.* (1995) redetermined the crystal structure of the α -form of DL-norleucine at 296 K. As it was usual at that time, a diffractometer with a point detector was used. However, this set-up makes it difficult to observe diffuse scattering. Correspondingly, diffuse scattering or disorder were not taken into account. The same authors also recorded synchrotron radiation Laue diffraction patterns of the α -phase over a range of 298–391 K. At 298 K they observed diffuse streaks along c^* , which is consistent with the observations of Mathieson (1953). However, the Laue pattern did not show the superstructure reflections with $c^{*'} = \frac{1}{4}c^*$, which were reported by Mathieson (1953). The diffuse streaks diminished greatly as the temperature approached 373 K leaving only the reflections of the α -phase. Hence, the disorder in the α -phase is reduced at higher temperatures.

The crystal structure of the β -phase of DL-norleucine was determined at 120 K by Dalhus & Görbitz (1996). The space group was given as $C2/c$, with cell parameters of $a = 31.067$ (5), $b = 4.717$ (1), $c = 9.851$ (2) Å, $\beta = 91.37$ (2)°. After transformation with $a' = -c$, $b' = b$, $c' = a + b$ the space group is $I2/a$ and cell parameters change to $a = 9.851$ (2), $b = 4.717$ (1), $c = 32.366$ (5) Å, $\beta = 106.35$ (2)°. Lattice parameters and atomic coordinates are in good agreement with the structure of the idealized β -phase suggested by Mathieson (1953), which confirms the suggested stacking sequence L–M–L–M. Again, the measurement of Dalhus & Görbitz was carried out with a

Table 1

Unit-cell volume of the structures calculated by DFT-D.

For better comparison all structures are given in the $P2_1/a$ and $I2/a$ settings of the unit cell.

Phase	α	α	β	$\beta^\dagger$	β	Disordered	γ	γ
Reference	Harding <i>et al.</i> (1995)	This work	Dalhus & Görbitz (1996)	This work	This work	This work	Coles <i>et al.</i> (2009)	This work
Method	Single crystal	DFT-D	Single crystal	Single crystal	DFT-D	Single crystal	Single crystal	DFT-D
Temperature (K)	296 (2)	—	120 (2)	173 (2)	—	293 (2)	395	—
Space group	$P2_1/a$	$P2_1/a$	$I2/a$	$I2/a$	$I2/a$	Disordered	$I2/a\ddagger$	$I2/a$
a (Å)	9.9069 (13)	9.80	9.851 (2)	9.8739 (5)	9.79	9.92290 (8)	9.8356 (7)	9.82
b (Å)	4.737 (2)	4.66	4.717 (1)	4.7296 (4)	4.66	4.73734 (4)	4.819 (3)	4.64
c (Å)	16.382 (2)	15.87	32.366 (1)	32.4710 (19)	31.69	16.3737 (3)§	33.697 (3)	32.61
β (°)	104.681 (11)	108.80	106.345 (2)	105.976 (5)	109.85	104.2600 (3)§	95.5924 (3)	87.22
Unit-cell volume (Å ³)	743.8 (3)	685.5	1443.2 (5)	1457.82 (17)	1360.8	745.98 (2)§	1589.8 (2)	1483.3
Z, Z'	4, 1	4, 1	8, 1	8, 1	8, 1	—	8, 1	8, 1

 † Affected by stacking disorder. § $P2_1/a$ setting. ‡ Transformed from the $C2/c$ setting of the original paper.

point detector and the crystal was apparently not investigated for diffuse scattering. Hence, it is not clear if the β -phase is fully ordered at 120 K, or exhibits a certain degree of stacking disorder. In the case of DL-methionine the α - and β -phases are fully ordered.

The molecular conformation of DL-norleucine is similar in the α - and β -phases and corresponds to the conformation of DL-methionine in its β -phase. In contrast, the conformation of DL-methionine in its α -phase is different. In α -methionine the side chain exhibits a *gauche* conformation [$\tau(\text{C3}—\text{C4}—\text{S5}—\text{C6}) = 68.9^\circ$] (Taniguchi *et al.*, 1980), whereas a *trans* conformation ($\tau \simeq 175^\circ$) is found for β -methionine, and α - and β -norleucine.

The crystal structure of the γ -phase of DL-norleucine was determined by Coles *et al.* (2009). The space group and hydrogen-bond pattern are the same as for the β -phase, but the molecular conformation is different. The torsion angle $\tau(\text{N}—\text{C2}—\text{C3}—\text{C4})$ is 171.9 (6)° in the γ -phase, but 56.5 (3)° and 52.7 (2)° in the α - and β -phases respectively. The crystallographic data of all three phases are compiled in Table 1.

All phases of DL-norleucine have similar lattice parameters a and b , which reflects the similarity of the hydrogen-bond networks within the double layers parallel to (001) in the three phases. The doubling of the c axis with a body-centring of the unit cell in the β - and γ -phases is a result of the different stacking sequences (L–M–L–M instead of L–L–L–L, see Fig. 2).

The phase transformation from the β - to the α -phase of norleucine was investigated by Anwar *et al.* (2007) by means of Molecular Dynamics (MD) simulations using the Williams force field and a model containing four double layers. These MD simulations revealed that the molecules of an entire double layer shift together. However, the observed shift was $(0, \frac{1}{2}, 0)$, i.e. by $\mathbf{b}/2$ and not $(\frac{1}{2}, \frac{1}{2}, 0)$ as would be required for the transition from the β - to the α -phase.

Zahn & Anwar (2013) investigated the phase transition by MD using transition path sampling. This method confirmed the shifting of entire double layers. In contrast to the previous

work, the double layer shifted by $(\frac{1}{2}, \frac{1}{2}, 0)$. As seen in the calculations of Anwar *et al.* (2007), this process did not occur simultaneously in the whole crystal but initiates with a shift of one double layer only. In the simulation box containing four double layers, the structure changed from L–M–L–M (β -phase) to L–L–L–M. The resulting sequence, L–L–L–M, corresponds to one of the superstructures observed by Mathieson (1953). The second transition, from L–L–L–M to L–L–L–L was apparently not observed within the calculation times of the MD simulation.

Recently, MD simulations were performed using the Amber94 force field, with a simulation box containing four double layers (van den Ende & Cuppen, 2014). As in the simulations of Anwar *et al.* (2007) and Zahn & Anwar (2013), the simulations showed a shift of an entire double layer, which changes the mutual arrangement of the moving double layer both with the right and the left neighbouring double layers. Additionally, the calculations revealed that the shift may also occur only on one interface. However, the MD simulations produced again only shifts by $(0, \frac{1}{2}, 0)$, and not by $(\frac{1}{2}, \frac{1}{2}, 0)$ as observed experimentally.

In 2015, van den Ende *et al.* (2015) performed MD simulations using the Amber94 force field and nudged elastic band (NEB) calculations. In a simulation box containing four double layers using MD simulations these authors again observed a partial phase transition induced by a shift of a double layer by $(0, \frac{1}{2}, 0)$. A full phase transition was not observed. From the NEB calculations the authors obtained the energy transition barriers of double-layer shifts, to prove which transition pathway is the most probable. In contrast to the results of the MD simulations they concluded that the most probable mechanism of the transition from the β - to the α -phase is first a sliding by $\mathbf{a}/2$, followed by a shift of $\mathbf{b}/2$. They suggested intermediate structures containing double layers of type A (shifted by $\mathbf{a}/2$ against L) and B (shifted by $\mathbf{b}/2$ against L), which have not been observed experimentally. The calculated transition barriers for the shift of these metastable A or B layers into the more stable L or M layers are relatively

low. Consequently the lifetime of an A or a B layer between L and M layers should be quite short, which would explain why the A and the B layers have not been detected experimentally.

Disorder in molecular crystals is no rarity. More than 212 000 crystal structures out of the 869 000 structures in the Cambridge Structural Database (Groom *et al.*, 2016) exhibit some type of disorder. Disorder occurs if at least two energetically similar molecular conformations or arrangements exist in the solid state. The packing energy of these different molecular conformations and arrangements can be calculated by lattice-energy minimizations. These provide information about the preferred local arrangement and distortions of the molecules raised by the position of neighbouring molecules. In most cases the disorder affects a small part of the molecule only. Different types of disorder are known, *e.g.* rotational disorder of side groups (*e.g.* alkyl chains) or orientational disorder of the entire molecule [*e.g.* SF₆ (Dove *et al.*, 2002) or Si[Si(CH₃)₃]₄ (Dinnebier *et al.*, 1999)]. Layered structures tend to form stacking disorder. Stacking disorder is a well known phenomenon in inorganic crystals such as graphite or ZnS, but is also observed in molecular crystals. Examples include the β -phase of Pigment Red 170 (Teteruk *et al.*, 2014), tris-(bicyclo[2.1.1]hexeneno)benzene (Schmidt & Glinnemann, 2012), form II of aspirin (Bond *et al.*, 2007*a,b*; Chan *et al.*, 2010) and eniluracil (Copley *et al.*, 2008). In the case of a stacking disorder, the molecular arrangement is well ordered in two dimensions, but disordered in the third dimension. The orientation of the molecules is given, but their position is disordered. In a diffraction pattern, such a stacking disorder results in diffuse streaks perpendicular to the layers.

In this work we analysed the local symmetry of the molecular layers in DL-norleucine to derive the possible stacking sequences. In order to investigate the origin of the stacking disorder, we perform lattice-energy minimizations on a variety of structural models with different stacking sequences using periodic force-field and density functional methods.

Subsequently stacking probabilities are derived using the Boltzmann formula. In order to simulate the diffuse scattering, models with large supercells are constructed, following the calculated stacking probabilities. The simulated diffraction pattern of these models should show the diffuse scattering. This approach worked very well for the β -phase of Pigment Red 170 (C₂₆H₂₂N₄O₄) (Teteruk *et al.*, 2014), and was also applied here. In addition, we performed single-crystal X-ray analysis on crystals of DL-norleucine at 173 and 293 K. The diffuse scattering was recorded and compared with the simulated diffraction patterns of the disordered models.

2. Symmetry analysis

Crystal structures with different stacking sequences of equivalent layers are called polytypes, which are a special kind of polymorph. Symmetry operations connecting the layers permit the build-up of extended ordered and disordered layer sequences.

2.1. Layer symmetry

In the crystal structures of α - and β -norleucine, a single layer of molecules can be described by a unit cell with basis vectors **a**₀ and **b**₀, corresponding to the unit-cell vectors **a** and **b** of the crystal structures of α - and β -norleucine in the $P2_1/a$ and $I2/a$ settings, respectively. The unit cell of a single layer contains two molecules. Both molecules point with their aliphatic chains to one side of the layer and with their charged groups to the other side. A single layer is thus crystallographically polar in the stacking direction.

Within the layers symmetrically equivalent molecules are related by *a*-glide planes perpendicular to **b** (see Fig. 3*a*). Thus the layer-group symbol of a single layer is $p1a1$, which is a non-standard setting of layer group $pb11$ (No. 12) (Kopský & Litvin, 2002); in both settings the stacking direction is **c**.

2.2. Double layers

Two adjacent single layers are connected by hydrogen bonds between the charged NH₃⁺ and COO[−] groups, forming head-to-head double layers along the stacking direction **c**. The second single layer is generated from the first single layer by inversion centres or 2₁ screw axes parallel to (010) (Fig. 3*a*). The resulting double layer has layer-group symmetry $p12_1/a1$, which is a non-standard setting of layer group $p2_1/b11$ (No. 17). The basis vectors of the unit cell of the double layer are **a**₀, **b**₀ and **c**₀, where **c**₀ corresponds to the unit-cell vector **c** in the $P2_1/a$ setting of the crystal structure of α -norleucine. For the origin of the unit cell we chose, according to the IUCr layer-group standard, the inversion centre in the middle of the double layer, *i.e.* between the charged groups of the molecules (Wilson & Hahn, 1995).

2.3. Stacking of double layers

The double layers are stacked along **c**₀. Neighbouring double layers are connected by either 2₁ screw axes parallel to [010] or by pure twofold rotation axes parallel to [010]. In both cases, the combination of the symmetry elements within the double layers and between them produces additional symmetry elements connecting the two double layers: inversion centres, *n*- or *c*-glide planes and translation [Figs. 3(*b*) and 3(*c*)]. A stacking using the 2₁ screw axis generates two double layers with the motif L–L (or M–M), whereas the application of the twofold rotation axis leads to L–M (or M–L).

The most ordered polytypes are generated by repeated application of only one type of symmetry operation. If the twofold screw axis is chosen throughout to generate the next double layers, a periodic structure with the sequence LL (= LLLL) arises, which is the α -phase (Fig. 3*b*). Exclusive application of the twofold rotation yields the periodic sequence LM (= LMLM), which is the β -phase (Fig. 3*c*). Combinations of 2 and 2₁ axes result in further periodic and non-periodic sequences including the periodic sequences LLMM and LLLM.

Layer structures with stacking disorder consisting of equivalent layers can be described with the order–disorder (OD) theory (Dornberger-Schiff & Fichtner, 1972; Dorn-

Figure 3

Models of DL-norleucine from the symmetry analysis. (a) Basic layer used for the symmetry analysis approach, (b) α -phase, (c) β -phase, (d) and (e) hypothetical stackings. Blue: unit cell of the double layer. Black: supercells from the symmetry analysis. Red: unit cells used for DFT-D calculations. The numbers denote the fractional y-coordinates of the asymmetric carbon atoms.

berger-Schiff 1982; Fichtner, 1988; Ferraris *et al.*, 2008). On first glance all double layers seem to be equivalent. However, the OD theory requires the geometrical equivalence of layer pairs (here: pairs of double layers). But actually there are two different pairs of double layers: those constructed by a 2_1 screw axis and those constructed by a twofold rotation axis. Hence the layers are not geometrically equivalent. The different sequences do not belong to the same OD family. The

α - and β -phases of norleucine are just different polytypes such as, for example, the forms I and II of aspirin (Bond *et al.*, 2007).

The γ -phase exhibits a different molecular conformation. Nevertheless, the symmetry elements within the double layers are the same as in the α - and β -phases, but neighbouring double layers are connected exclusively by twofold rotation axes, which results in the same molecular packing as seen in the β -phase.

2.4. Sequences including layers A or B

A disordered crystal of norleucine could not only contain double layers of type L and M but, at least theoretically, also double layers which deviate from an L layer by a lateral shift of $a/2$ (A layer) or $b/2$ (B layer). Such A and B layers were observed in the MD simulations (Anwar *et al.*, 2007; van den Ende & Cuppen, 2014; van den Ende *et al.*, 2015). In a stacking, an A layer is generated from a preceding L layer by a 2_1 screw axis parallel to b . The position of this 2_1 screw axis in an L–A sequence differs from the position of the 2_1 screw axis in an L–L sequence by $a/4$ [see Figs. 3(d) and 3(b)]. Similarly, a B layer is generated from a preceding L layer by a twofold rotation axis. The position of this twofold axis differs from that in an L–M sequence by $a/4$, too [see Figs. 3(e) and 3(c)]. As in L–L and L–M sequences, there are additionally inversion centres between double layers in L–A and L–B sequences.

The inclusion of layers A or B leads to two most-ordered polytypes, which are generated by repeated application of only one type of symmetry operation. The sequences LA (Fig. 3d) and LB (Fig. 3e) are generated by repeated application of 2_1 screw axes and twofold rotation axes, respectively. The structure LA has space group $B2_1/e$, which is a non-standard setting of $P2_1/c$. The space group of LB is $A2/a$, which is a non-standard setting of $C2/c$. Both structures have $Z' = 1$. All other structures, e.g. LAMB, have $Z' > 1$.

3. Computational and experimental details

3.1. Model building

Lattice-energy minimizations were performed on ordered model structures with large unit cells. All models were constructed based on the unit cell of the α -phase. This reference cell had lattice parameters of $a_0 = 9.9069$, $b_0 = 4.737$, $c_0 = 16.382$ Å, $\alpha = \gamma = 90^\circ$, $\beta = 104.681^\circ$ with the stacking in the c direction. For the superstructures with a larger unit cell, a corresponding multiple value of c_0 was used, e.g. $c' = 4c_0$ for LLMM.

Starting from a double layer L, a subsequent double layer L is generated by a translation of c_0 , a double layer M by $a_0/2 + b_0/2 + c_0$, a double layer A by $a_0/2 + c_0$ and a double layer B by $b_0/2 + c_0$. The resulting superstructures have either monoclinic or triclinic symmetry.

All models can be set up in different ways. For example, the stacking sequence LL is symmetry equivalent to MM, AA and BB. Similarly LM is symmetry equivalent to ML, AB and BA.

Correspondingly, the sequence LL, as well as the sequence LM, has a degeneracy of $g = 4$.

3.2. Force-field calculations

Lattice-energy minimizations with force-field methods were performed with the software package *Materials Studio* (Accelrys, 2008), using the Dreiding force field (Mayo *et al.*, 1990). The dispersion interactions were calculated by the 6-exp potentials suggested by Mayo *et al.* (1990). Atomic charges were obtained by the method of Gasteiger & Marsili (1980). Long-range electrostatic interactions were calculated using Ewald summation (Ewald, 1921) with a precision of 4×10^{-5} kJ mol⁻¹. The lattice-energy minimizations were performed in two steps. In the first step, all atomic positions were optimized with fixed lattice parameters. In the second step, the lattice parameters were optimized together with the atomic positions.

For the minimizations the SMART algorithm was used. The convergence criteria for energies, forces, cell stress and Cartesian displacements were 8×10^{-5} kJ mol⁻¹ atom⁻¹, 4×10^{-3} kJ mol⁻¹ Å⁻¹, 0.01 kbar and 0.00001 Å, respectively. Test calculations on the α - and β -phases showed that the crystal structures distorted slightly. The applied force field describes both phases of DL-norleucine in a reliable way, albeit exhibiting a shortening of 6% in the a axes for both phases. All models were calculated in the smallest unit cell. For ease of comparison and to identify possible distortions, all models were subsequently transformed to the setting of the reference unit cell.

3.3. DFT-D calculations

To obtain more accurate energies, additional lattice-energy minimizations were performed using density functional theory with dispersion correction (DFT-D). The *CASTEP* code (Academic release 6.1, Clark *et al.*, 2005) was used with the Perdew–Burke–Ernzerhof (PBE) functional (Perdew *et al.*, 1996) in combination with the semi-empirical dispersion correction by Grimme (2006). A plane-wave basis set cut-off of 520 eV and a k -point spacing of approximately 0.04 Å⁻¹ were applied. The convergence criteria for energies, forces, cell stress and Cartesian displacements were 0.001 kJ mol⁻¹ atom⁻¹, 3 kJ mol⁻¹ Å⁻¹, 0.5 kbar and 0.001 Å, respectively. The DFT-D minimizations were performed using the same two steps as used in the force-field minimizations. In the first step the lattice parameters were kept fixed and in the second step the atomic coordinates and lattice parameters were minimized simultaneously. Symmetry was imposed in all DFT-D minimizations. As in the force-field calculations, the energies are given per molecule.

3.4. Crystallization

DL-Norleucine was purchased from Alfa Aesar. Single crystals were obtained as thin plates from an ethanol–water solution by slow evaporation at room temperature over several weeks (Fig. 4). The largest observed single-crystal has a size of approximately 1.0 mm $\times$ 0.5 mm.

3.5. Single-crystal structure determination and search for diffuse scattering

A single crystal was rapidly cooled to 173 K, whereby it transformed to the β -phase. Diffraction data of this crystal were collected at 173 K on a Stoe-IPDS II diffractometer equipped with a GENIX micro-focus source and mirror optics. For reconstruction of the reciprocal space the *X-Area* software package (Stoe & Cie, 2001) was used. Subsequently, the crystal was reheated to room temperature and investigated on a Bruker SMART three-circle diffractometer with an Incoatec I μ S copper micro-focus source, mirror optics and an APEX2 CCD detector. The data collection, cell-refinement and data reduction were performed with the *APEX3* software (Bruker, 2012).

The structure of the β -phase at 173 K was solved by direct methods using the program *SHELXS* (Sheldrick, 2008) and refined against F^2 with full-matrix least-squares techniques using the program *SHELXL* (Sheldrick, 2015). The site occupation factor of the major occupied position refined to 0.950 (4). The minor occupied atoms were refined isotropically. H atoms bonded to C were refined using a riding model. H atoms bonded to N were refined isotropically. For the minor occupied position, H atoms could not be included (not even as riding), because several atoms had nonpositive definite displacement parameters, when these H atoms were added.

4. Results

4.1. Lattice-energies of different structural models

4.1.1. Lattice-energy minimizations using force fields. The results of the lattice-energy minimizations with models containing two double layers ($\mathbf{c}' = 2\mathbf{c}_0$) are shown in Table 2. Within the force-field approach the model LL, which corresponds to the α -phase, has nearly the same energy as the model LM, which corresponds to the β -phase. Experimentally the β -phase is the energetically preferred one at low

Figure 4
Single-crystals of DL-norleucine obtained from an ethanol–water solution by slow evaporation at room temperature.

Table 2

Results of the lattice-energy minimizations on selected models containing two ($c' = 2c_0$, $Z = 8$) and four double layers ($c' = 4c_0$, $Z = 16$).

Further models containing a mixture of L, M, A and B layers (e.g. LMAA) were minimized with force fields only (see Table S3 in supporting information).

Stacking sequence			Force-field methods	DFT-D	Remarks
Two double layers	Four double layers	Space group†	Relative energy (kJ mol ⁻¹)	Relative energy (kJ mol ⁻¹)	
LL	LLLL	$P2_1/a$, $Z = 4$	0.00	1.03	α -phase
	LLLM	$P2_1/a$, $Z = 16$	0.02	0.46	
LM	LMLM	$C2/c$, $Z = 8$	0.05	0.00	β -phase
	LLMM	$A2/a$, $Z = 16$	0.02	0.46	
LA	LLLA	$P2_1/a$, $Z = 16$	1.03	2.11	
	LALA	$P2_1/c$, $Z = 4$	2.01	4.19	
	LLAA	$P2_1/a$, $Z = 8$	1.03	3.19	
LB	LLLB	$P2_1/a$, $Z = 16$	0.99	2.11	
	LBLB	$A2/n$, $Z = 8$	1.92	4.19	
	LLBB	$A2/a$, $Z = 16$	0.99	2.67	
	LMAA	$P2_1/a$, $Z = 8$	1.01	2.61	
	LMAM	$P2_1/a$, $Z = 16$	1.01	1.73	
	LMBM	$P2_1/a$, $Z = 16$	1.05	1.71	

† Space group of the model used for DFT-D.

temperature, whereas the α -phase is the more stable one at higher temperatures.

Stacking sequences containing a mixture of L layers with A or B layers are energetically less favourable by about 2 kJ mol⁻¹. Hence a mixture of L (or M) with A or B double layers should be rare. The same tendency can be seen in force-field calculations containing models with four double layers ($c' = 4c_0$), which are included in Table 2.

Within the models containing four double layers, all sequences built from only L and M double layers are preferred and have nearly the same energy. Every transition from L or M to A or B increases the energy by about 2 kJ mol⁻¹. Models that have a mixture of local stacking motifs, e.g. LLBB, possess energies which are close to the average of the corresponding individual stackings. For example, the energy of LLBB (0.99 kJ mol⁻¹) is very close to the average of LL (0.00 kJ mol⁻¹) and LB (1.92 kJ mol⁻¹). This indicates that the energy of a double layer depends almost exclusively on the arrangement of the neighbouring layers only, whereas interactions with more distant layers do not play a major role.

4.1.2. Lattice-energy minimizations using DFT-D. Selected models with two or four double layers were optimized using DFT-D. Within the models containing two double layers, the energy of the stacking sequence LM (which corresponds to the β -phase) is lower, by 1.03 kJ mol⁻¹, than that of the sequence LL (α -phase), which is in agreement with the experimentally observed stability order at low temperature. (The DFT-D calculations do not include any entropic contribution, whereas the force field has been parameterized using room-temperature structures.)

The energy difference from the DFT-D calculations corresponds well with the value of 1.2 kJ mol⁻¹ calculated by van

den Ende *et al.* (2015) using the Amber force field with AM1-BCC charges.

The stacking sequences LA and LB are significantly less favourable, with an energy increase of 4.19 kJ mol⁻¹.

For the models containing four double layers ($c' = 4c_0$, $Z = 16$) with 352 atoms per unit cell the DFT-D calculations are quite time consuming at present. Therefore, only selected models were calculated. The results are included in Table 2. The stacking sequence LMLM, which corresponds to the β -phase, is the energetically preferred one. The subsequent energy rankings are found for LLMM ($\Delta E = 0.46$ kJ mol⁻¹) and LLLM ($\Delta E = 0.46$ kJ mol⁻¹). Both structures have been proposed as models for the disordered structure of the β -phase by Mathieson (1953). The energy of the α -phase is even higher ($\Delta E = 1.03$ kJ mol⁻¹), which confirms that the formation of the α -phase is only driven by entropy. However, the energy differences between all structures built from L and M layers are relatively low, which causes the stacking disorder experimentally observed as diffuse streaks.

All models containing A or B layers are considerably higher in energy. Stacking a double layer A or B onto L or M increases the energy by at least 3.4 kJ mol⁻¹. For example, the periodic structure LMBM contains four different local stackings (LM, MB, BM and ML). LM is symmetrically equivalent to ML, MB is symmetrically equivalent to BM. The local stackings LM and ML are energetically favourable. The other two have higher energies. Assuming that the LM and ML stackings have a relative energy of 0.00 kJ mol⁻¹, the average energy of the model LMBM of 1.71 kJ mol⁻¹ leads to the conclusion that the stackings MB and BM increase the energy by 3.42 kJ mol⁻¹ each.

Kinetically, MD calculations with force fields (van den Ende *et al.*, 2015) showed that neighbouring double layers can quite easily shift against each other. Consequently mixed L/M/A/B sequences are neither thermodynamically favourable nor substantially kinetically stabilized. In a real crystal such sequences cannot be fully excluded, but their occurrence should be rare and can be considered as a lattice defect.

4.2. On the mechanism of the phase transition $\beta \rightarrow \alpha$

For the phase transition from the β - to the α -phase MD calculations with force fields suggested that the phase transitions starts with a shift of a double layer along the *a* or *b* axes, e.g. from LMLM to LMAM or from LMLM to LMBM, because of low transition energy barriers and small energy differences between LMLM and LMAM or LMBM. Our calculations make no statement about the energy barriers, but the resulting structures LMAM or LMBM are energetically very unfavourable with an energy increase of at least 6–7 kJ mol⁻¹. The DFT-D calculations suggest that the shift should occur directly from LMLM to LMMM, without LMAM or LMBM as intermediate structures (see Fig. 5, path 1). Similarly, a diagonal shift of a block of two (or more) double layers may occur, see Fig. 5, path 2. Alternatively the transition may start with a shift on one interface only. van den Ende's MD calculations (van den Ende *et al.*, 2015) proposed

such a shift in **b** direction (Fig. 5, path 3), but DFT-D calculations suggest a diagonal shift instead (Fig. 5, path 4). Hence, static DFT-D calculations suggest paths 1, 2 or 4, whereas MD calculations with force fields suggest path 3. In the future, this question might be solved by MD calculations with DFT-D, but this is beyond the scope of the present paper.

4.3. Stacking probabilities

The lattice energies of the various models can be used to derive the stacking probabilities (Teteruk *et al.*, 2014). From the DFT-D energies of the models, we calculated the probability of finding a specific layer triple, *e.g.* three subsequent double layers with the stacking sequence ... MLM ..., in a non-periodic infinite stacking sequence consisting of L and M double layers (for details see the supporting information). The resulting stacking probabilities are 4.4% for ... LLL ... or ... MMM ..., 23.3% for ... LML ... or ... MLM ... and 11.1% for the remaining layer triples (... LLM ...,

... MML ..., ... LMM ... or ... MLL ...). Stackings with A or B layers have negligible probabilities and were, therefore, omitted in further calculations.

4.4. Simulation of diffuse scattering

The stacking probabilities were used to construct a large model, containing 100 double layers with the sequence LMLMMLMMMLMLMMLMLLMLMLLMLMLMMLMLMMLMLLMLMLMMLLMLMLLLLLMLMLLMLMLMLMMLLMLMLMLMLMLMLLMLMLMMLMMLMMLMMMLMMLMML. This model contains 8800 atoms and has lattice parameters of $a = 9.9069$, $b = 4.7370$, $c = 1638.2$ Å, $\beta = 104.6810^\circ$. The simulated diffraction pattern of this model has reflections between a pair of Bragg reflections parallel to the c^* direction, which is a good representation of the diffuse scattering. The simulated diffraction pattern shows sharp intensity maxima for reflections with $h + k = 2n$ and pronounced diffuse streaks parallel to the c^* axis, through all reflections with $h + k = 2n + 1$ (see Fig. 6a).

4.5. Single-crystal X-ray analyses

The investigated single crystal, grown at room temperature, turned out to be the α -phase, as revealed by single-crystal X-ray analysis. This crystal was rapidly cooled to 173 K. At 173 K, the experimental diffraction pattern (Fig. 6c) consists of sharp reflections of the β -phase (with *I* centring), weak reflections violating the *I* centring at $h + k = 2n + 1$, $l = 2n$, and weak diffuse streaks parallel to the c^* axis through all reflections with $h + k = 2n + 1$. The position and orientation of the diffuse streaks agree with our calculations for structures built by L and M layers, and with the observations of Mathieson (1953) and Harding *et al.* (1995) at room temperature. Apparently, the β -phase at 173 K is still affected by the stacking disorder. Neglecting the diffuse streaks and the weak reflections violating the *I* centring, the structure of the β -phase was redetermined from the data measured at 173 K. A careful analysis of the residual electron density revealed a second

Figure 5
Possible pathways for the start of the phase transition from the β - to the α -phase of DL-norleucine. Path 1: an individual double layer is shifting in diagonal direction. Path 2: a block of two (or more) double layers are shifting in diagonal direction. Path 3: consecutive shifts of double layers in *a* and *b* direction (or *vice versa*) on one interface only. Path 4: a shift in diagonal direction on one interface only. DFT-D calculations suggest paths 1, 2 or 4.

Figure 6
Simulated and experimental single-crystal diffraction patterns of norleucine, $0kl$ layer. (a) Simulated pattern of a model containing 100 double layers with a stacking sequence of L and M layers according to the stacking probabilities calculated by DFT-D. The intensity is represented by the size of the circles. (b) Experimental pattern of the disordered phase of DL-norleucine at 293 K. In (a) and (b) the arrows denote the vectors corresponding to the unit-cell setting $P2_1/a$. (c) Experimental pattern of the disordered β -phase of DL-norleucine at 173 K, showing the disordered β -phase ($I2/a$, $c' = 2c$).

molecular position with an occupancy of 0.050 (4) (see Fig. 7). The major occupied atomic positions agree very well with the structure of the β -phase determined by Harding *et al.* (1995). The minor occupied molecular positions agree well with an occurrence of ...LL... (or ...MM...) instead of ...LM... stacking. The strong sharp reflections of the $I2/a$ cell show that the crystal mainly consists of quite large and at least quite well ordered domains of LMLM sequence (β -phase). The occurrence of weak sharp reflections at $h + k = 2n + 1$, $l = 2n$ points to the existence of a small number of at least quite well ordered domains of LLLL sequence (α -phase), which are sufficiently large to cause such sharp reflections. Additionally, the diffuse scattering points to stacking faults within these domains and/or the existence of less ordered regions.

After reheating the investigated crystal from 173 K to room temperature, the diffraction pattern is considerably different (Fig. 6b). Reflections with $h + k = 2n$ are still sharp. At the positions of the reflections with $h + k = 2n + 1$, there are very intense diffuse streaks parallel to the c^* axis. The diffuse streaks are continuous, but show intensity maxima at $l = 2n + 1$ and weaker maxima at $l = 2n$. The picture is in qualitative agreement with the simulated diffraction pattern, calculated from the disordered structure with 100 layers of L and M. A structural model which includes also A and B layers would cause diffuse streaks parallel to c^* at $h + k = 2n$, but the experimental pattern at room temperature did not show any trace of diffuse scattering at these positions. Hence, the experiment disproves the existence of a significant amount of A and B layers in disordered norleucine.

A second single crystal was grown under the same conditions as the first one. This crystal was rapidly cooled to 120 K. Surprisingly, no phase transition from the α - to the β -phase occurred and the crystal remained in the α -phase. The pattern did not contain any diffuse streaks. After reheating to room temperature the α -phase without diffuse streaks was still present. Apparently, the observed phases of DL-norleucine strongly depend on the 'temperature history' of the crystals.

Figure 7

Displacement ellipsoid plot of DL-norleucine at 173 K. Major occupied atomic positions are drawn as ellipsoids, minor occupied atomic positions as spheres. The ellipsoids are drawn at 50% probability level.

5. Conclusion

In this work we investigated the origin for the stacking disorder in DL-norleucine. Therefore, lattice-energy minimizations were performed on ordered models containing two and four double layers per unit cell, respectively, using force-field methods and dispersion-corrected density functional theory (DFT-D). Lattice energies obtained by these methods show that the structure of norleucine exhibits energetically preferred stacking sequences. The stacking sequence LMLM, which corresponds to the β -phase of DL-norleucine, is the energetically preferred one. This stacking sequence is energetically more favourable by 1.03 kJ mol^{-1} than the stacking sequence of the α -phase (LLLL), which is in good agreement with the stability order observed experimentally. Stacking sequences containing a mixture of only L and M layers, such as LLMM or LLLM suggested by Mathieson (1953), are energetically between the α - and β -phases. The energy differences between all structures, built from L and M layers, are relatively low: this gives rise to the stacking disorder, which is observed experimentally as diffuse streaks.

A mixture of L and M with A and B layers is energetically less favourable: every transition from an L or an M layer to A or B increases the energy by at least 3.4 kJ mol^{-1} . Therefore A and B layers are very rare.

Stacking probabilities were calculated with Boltzmann statistics. Using these probabilities, a large model containing 100 double layers with a stacking sequence of L and M layers was constructed. This model was used to simulate single-crystal diffraction patterns, which reproduce strong diffuse streaks parallel to the c^* axis through all reflections with $h + k = 2n + 1$.

Experimental investigations on single-crystals revealed that at 173 K norleucine exists in the β -phase with stacking disorder. After reheating to room temperature, we found a severely disordered structure built of L and M layers. The reflections with $h + k = 2n$ were free of any diffuse scattering, which revealed that the crystal does not contain a detectable amount of either A or B layers. Hence, the experimental single-crystal pattern at room temperature is in good agreement with the lattice-energy minimizations using DFT-D.

6. Related literature

References cited in the supporting information include: Dalhus & Görbitz (1996), Harding *et al.* (1995), van de Streek & Neumann (2010).

Acknowledgements

The authors thank Stefan Habermehl (Goethe-Universität, Frankfurt am Main) for simulation of the diffraction patterns, Lothar Fink (Goethe-Universität, Frankfurt am Main) for measuring the second single crystal, Jacco van de Streek (Avant-Garde Materials Simulations, formerly at Copenhagen University) for helpful discussions and an anonymous referee for valuable remarks on the symmetry analysis.

Funding information

The authors gratefully acknowledge the Villum Foundation (Denmark) for financial support (project No. VKR023111 of Jacco van de Streek).

References

- Accelrys (2008). *Materials Studio*. Release Notes. Accelrys Software Inc., San Diego, USA.
- Anwar, J., Tuble, S. C. & Kendrick, J. (2007). *J. Am. Chem. Soc.* **129**, 2542–2547.
- Bond, A. D., Boese, R. & Desiraju, G. R. (2007a). *Angew. Chem.* **119**, 621–624.
- Bond, A. D., Boese, R. & Desiraju, G. R. (2007b). *Angew. Chem.* **119**, 625–630.
- Bruker (2012). *APEX3*. Bruker AXS Inc., Madison, Wisconsin, USA.
- Chan, E. J., Welberry, T. R., Heerdegen, A. P. & Goossens, D. J. (2010). *Acta Cryst.* **B66**, 696–707.
- Cirino, P. C., Tang, Y., Takahashi, K., Tirrell, D. A. & Arnold, F. H. (2003). *Biotechnol. Bioeng.* **83**, 729–734.
- Clark, S. J., Segall, M. D., Pickard, C. J., Hasnip, P. J., Probert, M. I. J., Refson, K. & Payne, M. C. (2005). *Z. Kristallogr.* **220**, 567–570.
- Coles, S. J., Gelbrich, T., Griesser, U. J., Hursthouse, M. B., Pitak, M. & Threlfall, T. (2009). *Cryst. Growth Des.* **9**, 4610–4612.
- Copley, R. C. B., Barnett, S. A., Karamertzanis, P. G., Harris, K. D. M., Kariuki, B. M., Xu, M., Nickels, E. A., Lancaster, R. W. & Price, S. L. (2008). *Cryst. Growth Des.* **8**, 3474–3481.
- Dalhus, B. & Görbitz, C. H. (1996). *Acta Cryst.* **C52**, 1761–1764.
- Dalhus, B. & Görbitz, C. H. (2000). *Acta Cryst.* **B56**, 720–727.
- Dinnebier, R. E., Dollase, W. A., Helluy, X., Kümmerlen, J., Sebald, A., Schmidt, M. U., Pagola, S., Stephens, P. W. & van Smaalen, S. (1999). *Acta Cryst.* **B55**, 1014–1029.
- Dornberger-Schiff, K. (1982). *Acta Cryst.* **A38**, 483–491.
- Dornberger-Schiff, K. & Fichtner, K. (1972). *Krist. Techn.* **7**, 1035–1056.
- Dove, M. T., Tucker, M. G. & Keen, D. A. (2002). *Eur. J. Mineral.* **14**, 331–348.
- Ende, J. A. van den & Cuppen, H. M. (2014). *Cryst. Growth Des.* **14**, 3343–3351.
- Ende, J. A. van den, Smets, M. M. H., de Jong, D. T., Brugman, S. J. T., Ensing, B., Tinnemans, P. T., Meekes, H. & Cuppen, H. M. (2015). *Faraday Discuss.* **179**, 421–436.
- Ewald, P. P. (1921). *Ann. Phys.* **369**, 253–287.
- Ferraris, G., Makovicky, E. & Merlino, S. (2008). *Crystallography of Modular Materials*. Oxford University Press, UK.
- Fichtner, K. (1988). *Comput. Math. Appl.* **16**, 469–477.
- Gasteiger, J. & Marsili, M. (1980). *Tetrahedron*, **36**, 3219–3228.
- Görbitz, C. H. (2011). *J. Phys. Chem. B*, **115**, 2447–2453.
- Görbitz, C. H., Alebachew, F. & Petříček, V. (2012). *J. Phys. Chem. B*, **116**, 10715–10721.
- Görbitz, C. H., Vestli, K. & Orlando, R. (2009). *Acta Cryst.* **B65**, 393–400.
- Grimme, S. (2006). *J. Comput. Chem.* **27**, 1787–1799.
- Groom, C. R., Bruno, I. J., Lightfoot, M. P. & Ward, S. C. (2016). *Acta Cryst.* **B72**, 171–179.
- Harding, M. M., Kariuki, B. M., Williams, L. & Anwar, J. (1995). *Acta Cryst.* **B51**, 1059–1062.
- Ichikawa, T. & Itaka, Y. (1968). *Acta Cryst.* **B24**, 1488–1501.
- Kopský, V. & Litvin, D. B. (2002). *International Tables for Crystallography. Vol. E: Subperiodic groups*. Kluwer Academic Publishers, Dordrecht.
- Mathieson, A. McL. (1952). *Acta Cryst.* **5**, 332–341.
- Mathieson, A. McL. (1953). *Acta Cryst.* **6**, 399–403.
- Mayo, S. L., Olafson, B. D. & Goddard, W. A. III (1990). *J. Phys. Chem.* **94**, 8897–8909.
- Mnyukh, Y., Panfilova, N. A., Petropavlov, N. N. & Uchvatova, N. S. (1975). *J. Phys. Chem. Solids*, **36**, 127–144.
- Perdew, J. P., Burke, K. & Ernzerhof, M. (1996). *Phys. Rev. Lett.* **77**, 3865–3868.
- Schmidt, M. U. & Glinnemann, J. (2012). *Z. Kristallogr.* **227**, 805–818.
- Sheldrick, G. M. (2008). *Acta Cryst.* **A64**, 112–122.
- Sheldrick, G. M. (2015). *Acta Cryst.* **C71**, 3–8.
- Smets, M. M. H., Brugman, S. J. T., van Eck, E. R. H., van den Ende, J. A., Meekes, H. & Cuppen, H. M. (2015). *Cryst. Growth Des.* **15**, 5157–5167.
- STOE & Cie GmbH (2001). *X-Area*. STOE & Cie GmbH, Darmstadt, Germany.
- Streek, J. van de & Neumann, M. A. (2010). *Acta Cryst.* **B66**, 544–558.
- Taniguchi, T., Takaki, Y. & Sakurai, K. (1980). *Bull. Chem. Soc. Jpn*, **53**, 803–804.
- Teteruk, J. L., Glinnemann, J., Gorelik, T. E., Linden, A. & Schmidt, M. U. (2014). *Acta Cryst.* **B70**, 296–305.
- Tuble, S. C., Anwar, J. & Gale, J. D. (2004). *J. Am. Chem. Soc.* **126**, 396–405.
- Wilson, A. J. C. & Hahn, T. (1995). *International Tables for Crystallography. Vol. C: Mathematical, physical and chemical tables*. Dordrecht: Kluwer Academic Publishers.
- Zahn, D. & Anwar, J. (2013). *RSC Adv.* **3**, 12810–12815.