Disorderly Conduct of Benzamide IV: Crystallographic and Computational Analysis of High Entropy Polymorphs of Small Molecules

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ABSTRACT: Benzamide, a simple derivative of benzoic acid and a common intermediate of pharmaceutical compounds, was reported to form two polymorphs in 1832, but the single crystal structure of the more stable form was not solved until 1959. Nearly 50 years later, the second form was characterized by powder diffraction, followed shortly thereafter by characterization of a third form, a polytype of the most thermodynamically stable Form I. These two new forms, Forms II and III, are metastable. Herein, we describe a fourth polymorph, Form IV, discovered by melt crystallization concurrently with its crystallization under confinement at small length scales (<10 nm), where it is stable indefinitely. Form III exists under confinement in larger pores, and melting point data for different pore sizes corroborate the existence of Form IV below 10 nm. Form IV is highly disordered, precluding indexing of powder diffraction data other than hkl reflections. Nonetheless, a combination of powder X-ray diffraction and computational crystal structure prediction reveals that Form IV contains a 2D motif resembling that of Form II, but with long-range order in the third dimension masked by ubiquitous stacking faults. This approach relies on distilling a large number of candidate structures to a few possible disorder models based on benzamide tetrads that organize in 2D parquet-like tiles, with organization along the third dimension, that can be modeled with various stacking fault configurations having distinct intermolecular interactions and translations in the dimension orthogonal to the tiling planes. These observations reveal a bewildering crystallographic complexity for such a simple molecule. Nonetheless, the approach described herein demonstrates that challenging structures that may be abandoned prematurely because of poor crystallinity, twinning, or disorder can be solved.

INTRODUCTION

Wöhler and von Liebig, while struggling to make sense of constitution in organic compounds, cooled a boiling, aqueous solution of benzamide and recorded the remarkable transformation of silky needles into well-formed rhombic crystals.1 Thus, benzamide (Scheme 1), now recognized as a common intermediate for pharmaceutical compounds, became the first reported example of polymorphism of molecular crystals.2–5 The single crystal structure of the stable (rhombic) Form I was not solved until 1959 and was assigned as monoclinic.6 Nearly 50 years later, the transient Form II was prepared in situ as a mixture with I, and a structure was extracted from powder X-ray diffraction (PXRD) data.8,9 A third metastable polymorph (Form III), revealing a polytypic relationship to Form I, was characterized shortly afterward.10,11 Yet, another polymorph has been suggested by crystal structure prediction (CSP) but not found experimentally.12 The characterization of the metastable benzamide polymorphs is complicated further by the formation of bi- or triphasic mixtures, requiring extensive optimization of crystallization protocols to minimize secondary nucleation of Form I.11,13,14 Computational studies suggested an alternative structure for Form II, attributing the previous structure solution to insufficient data owing to the preferred orientations of needle-like crystals and the associated weak intensity of hkl reflections with l ≠ 0.15 Absent or weak
reflections, however, also can be explained by stacking disorder. While stacking faults are well-documented for minerals or synthetic zeolites,16–20 they are less frequently reported for molecular solids.21,22 The disorder unavoidably leads to poor quality or incomplete data, thereby frustrating structure solution using conventional characterization methods. Recently crystal structure prediction and density functional theory have emerged as powerful tools for overcoming limitations in small molecule structure determination23–26 and for providing further insight into molecular packing interactions.27,28 These reports, however, have not addressed stacking disorder in molecular solids.

We report herein a new metastable polymorph of benzamide. The new form, denoted here as Form IV, was discovered by melt crystallization using optical microscopy concurrent with its crystallization under nanofracture. It was confirmed as a unique polymorph by powder X-ray diffraction (PXRD), Raman spectroscopy, and differential scanning calorimetry. Melt crystallization or confinement produced Form IV with negligible amounts of contamination by other phases, enabling acquisition of powder data corresponding to a single phase. The PXRD data indicated a high degree of order in one dimension, but the absence of reflections other than hk0 suggested disorder in the perpendicular plane. Nonetheless, a combination of PXRD and crystal structure simulation reveals that Form IV contains a 2D motif resembling that of Form II, but with long-range order in the third dimension masked by ubiquitous stacking faults. This approach relies on distilling a large number of candidate structures to a few possible disorder models based on benzamide tetrad that organize in 2D parquet-like tiles, with organization along the third dimension, that can be modeled with various stacking fault configurations characterized by different intermolecular interactions and translations orthogonal to the parquet tiling planes. This combined experimental–computational approach demonstrates that challenging structures that may be abandoned prematurely because of poor crystallinity, twinning, or disorder can be solved.

### EXPERIMENTAL SECTION

**Materials and Methods.** Benzamide (C,H,N, 99%) was purchased from Sigma-Aldrich (St. Louis, Missouri) and was used without further purification. Controlled pore glass (CPG), a borate–silicate composite glass, from which the borate phase is leached to produce a silica glass bead with a random pore network, was obtained from Biosearch Technologies (Petaluma, CA) with nominal pore sizes of 35, 50, 100, and 200 nm. Porous glass with nominal pore sizes of 4 and 8 nm were purchased from Sigma-Aldrich. The CPG manufacturer specifications are provided in Table S1. All porous glasses were washed with nitric acid prior to use, then washed with deionized water and dried for 12 h under vacuum. The acid-washed matrices were stored under air in a desiccator.

**Crystal Growth.** Form IV was prepared by melting 2–4 mg of benzamide powder (mp = 125–128 °C) in the presence of natural resins between two glass coverslips on a Kofer bench and rapidly cooling to room temperature (ca. 20 °C). It has been suggested that the addition of resins, such as Canada balsam or gum mastic, can suppress crystal growth until high crystallization driving forces can be achieved at lower undercoolings.29 The amount of resin can vary, from 10 to 30 wt %, and in all cases spherulites corresponding to Form IV were observed. The porous glass beads were mixed with benzamide bulk crystals, heated above the benzamide Tm = 130 °C, and allowed to absorb the benzamide by capillary action for 2 min while heating in the DSC instrument. Cooling these nanoporous matrices below the melting point of benzamide results in benzamide nanocrystals embedded within the nanopores, as evident from Bragg peak assignments to benzamide in PXRD data collected on the matrices. Although the extent of pore filling has been reported to have minimal effect on the change of melting temperature,60,61 overfilling was avoided to prevent thermal signatures of bulk material. The mass ratio of benzamide and CPG and the manufacturer provided pore volume were used to calculate that 67–70% of the pores were filled. The heating and cooling of the benzamide–matrix mixtures were performed with a differential scanning calorimeter at 10 °C/min. Differential scanning calorimetry (DSC) was performed on a Pyris-1 (PerkinElmer Inc., Wellsley, Massachusetts) differential scanning calorimeter. An indium standard was used to calibrate the instrument, and nitrogen was used as the purge gas. Enthalpies were calculated by integrating the areas under the peaks after defining a baseline. Metastable II was prepared by evaporation of ethanol solution (140 mg/mL). The benzamide obtained commercially was confirmed by PXRD as Form I. Form III was obtained as the transformation product of Form IV formed from the melt.

**X-ray Diffraction.** Two-dimensional X-ray microdiffraction (2D µ-XRD) was performed with a Bruker D8 Discover General Area Detector Diffraction System (GADDS) equipped with a VANTEC-2000 2D detector and Cu Kα source (λ = 1.54178 Å). The X-ray beam was monochromated with a graphite crystal and collimated with a 0.5 mm capillary collimator (MONOCAP). Form IV was measured on a glass slide with the coverslip removed. CPG beads consisting of benzamide nanocrystals were loaded into a Kapton 124 capillary for measurement. High signal-to-noise ratio synchrotron powder diffraction data was collected for Form IV on the 17-BM beamline of the Advanced Photon Source, Argonne National Laboratory, at a wavelength of 0.45212 Å using a PerkinElmer PE1621 area detector. The sample–detector distance was 800 mm, and the measurement covered the angular range 2θ = 0.8–18.8°. A Kapton 1.0 mm capillary was loaded with benzamide powder, which was then melted by heating at ca. 150 °C (above Tm = 130 °C) then rapidly cooled down to 100 K using an Oxford 700+ Cooler and measured at the same temperature. High-resolution synchrotron powder diffraction data of Form IV were collected at beamline 11-BM of the Advanced Photon Source, Argonne National Laboratory, using a wavelength of 0.412827 Å. Discrete detectors covering an angular range from 6 to 16° 2θ were scanned over a 34° 2θ range, with data points collected every 0.001° 2θ and scan speed of 0.01°/s. A few milligrams of benzamide mixture with ca. 20% of gum mastic were placed between a microscope slide and a glass coverslip and melted on a Kofer bench at ca. 140 °C. The sample was rapidly cooled to room temperature. The coverslip was removed, and the powder of Form IV was carefully scraped with a needle. The powder was loaded into the Kapton 1.5 mm capillary and measured at 100 K using Oxford 700+ Cooler. The data collected on the two beamlines were in agreement, and data acquired on 11-BM did not add appreciably to the structure solution.

**Computational Methods.** CSP searches were performed using a rigid body random search algorithm in the UPACK program suite32 with each molecule described using the general Amber force field (GAFF).33 Atomic point charges for the classical force field were determined using the restricted electrostatic potential (RESP) charge assignment scheme34 using RHF/6-31G*/MP2/6-31G* in Gaussian 09.35 Individual searches were performed in parallel on selected space groups with a fixed number of molecules in the asymmetric unit (Z'). Z' = 1 structure searches included the space groups I41a, 421, 422, 12/c, Cs, P1, P21, P21, P212121, P21212, P21212, P21, P212, P21, P212121, P21, P21, Pbca, Pbcn, Pnca, Pca21, Pcss, and Pnma21. The Z' = 2 searches were performed for the space groups C2/c, Cc, P1, P21, P212, P212, P212, P212, Pbca, Pbcn, Pca21, Pcss, and Pnma21. The space groups tested are the most commonly occurring space groups for molecular crystals in the Crystal Structure Database. At least 500 structures were generated in each search, with up to 50,000 candidate structures generated for some space groups. This collection of structures was then subject to similarity clustering and flexible molecule optimization routines, resulting in 2551 unique crystal structures within 40.0 kJ/mol of the
lowest energy form. A summary of the CSP results and the resulting energy vs molecular volume plot highlighting the relevant predicted forms are provided in Figure S4. DFT optimizations were run in CP2K v. 2.6.2 using the Gaussian and plane wave (GPW) scheme of the QUICKSTEP package. In the first phase, cells were optimized with a cutoff of 450 Ry, the PBE exchange-correlation functional, and the DZVP-MOLOPT-GTH (m-DZVP) basis set paired with the appropriate dual-space GTH pseudopotentials. All supercells were at least 10 Å on all sides. In the second optimization phase, the D3(BJ) dispersion correction was performed with the active integration grid and the following QUICKSTEP variables: 5 grids with a cutoff of 900 Ry and a relative cutoff of 70 Ry, EPS_RHO = 1 × 10^-12; EPS_PGF_ORB = 1 × 10^-7. The space group for each optimized structure was determined using PLATON.

Diffuse-PXRD and (hkl) projections were calculated from our 20 × 20 × 1 unit cell atomistic crystal disorder models using the respective programs, DISCUS or DIFFUSE. Each short-range order (SRO) model output was controlled using the stochastic structure generator methods available in the program ZMC. Other ad hoc computational scripts were used to read in relevant preliminary crystal structure coordinates and then create the multiple coordinate “building-block” sets necessary as separate input files for the ZMC program. The powder module in DISCUS was run in complete integration mode using the pseudo-Voigt peak shape function with integration mode using the pseudo-Voigt peak shape function with a Lorentz correction parameter was manually adjusted to 13.27, and the hkl grid was evenly spaced at 1.00 reciprocal lattice units (r.l.u.). All other parameters are default. The hkl diffraction section was calculated by averaging “total” scattering intensities over 2 lots that comprised 10 × 10 × 1 unit cells.

The interface energies were calculated by building two blocks of each structure (6 × 3 × 6 unit cells for P2_1_2_2 structures, 6 × 2 × 6 unit cells for ac interfaces of Fdd2 structures, and 3 × 2 × 10 unit cells for bc interfaces of Fdd2 structures), which were combined to create smooth interfaces. The energies of such structures, E_inter, were calculated under 3D periodic boundary conditions. Optimization and MD simulations were performed using LAMMPS (Large-scale Atomic/Molecular Massively Parallel Simulator) with the same GAFF parameters used for CSP. All the MD simulations were 3 ns and were performed in the NPT_F (constant particle number, pressure, temperature with flexible cells) ensemble at 300 K and 1 atm with a 1 fs time step. The energy values for each block and the combinations were averaged over the last 500 frames. The energies of corresponding bulk P2_1_2_2 and Fdd2 structures (E_bulk) were calculated in the same way. The interface energies were calculated as E_interface = (E_inter - E_bulk)/A, where A is the area of the interface. Since the energies of superimposed structures evaluated are the same (only their configurations or orientations were different), there was no need to calculate energies for each of two original building blocks.

The crystal-adiabatic free energy dynamics (C-AFED) simulations were run for 0.5 ns using a modified version of the MD code platform PINY_MD. Simulation sizes were 4 × 2 × 8 unit cells (corresponding with a, b, and c) for the P2_1_2_2 and P2_2_1_2 structures (2 × 1 × 8 unit cells for the Fdd2), which produced a nearly cubic cell and total of 8192 atoms. Prior to the C-AFED run, each system was subject to energy optimization, as well as consecutive NVT and NPT_F equilibrations at 300 K for 20 ps. The time step for all MD runs was 0.5 fs. The C-AFED cell thermostat temperature was 200 000 K with the “mass-like” τ parameter set at 1000 fs.

### RESULTS AND DISCUSSION

#### Crystallization and Thermotropic Behavior

Melt crystallization is a powerful method for polymorph discovery as it creates conditions far from equilibrium, yet results in slow growth and nucleation. This has been demonstrated by our identification of new polymorphs grown from the melt for a wide range of materials, including paracetamol, testosterone propionate, aspirin, coumarin, resorcinol, and DDT. Crystal growth under nanoconfinement also has been used for the discovery and stabilization of metastable forms, wherein polymorph stability rankings deviate from bulk (unconfined) behavior.

Rapid cooling of a benzamide melt with gum mastic to room temperature (ca. 20 °C) yielded two kinds of spherulites with distinct morphologies (Figure 1). Powder X-ray diffraction and Raman spectroscopy revealed that one of these morphologies corresponded to Form III, as deduced from comparison with published and newly collected data for Forms I, II, and III. The other morphology, consisting of optically positive spherulites (large refractive index is radial), afforded a distinct PXRD pattern and Raman spectrum, supporting the formation of a new Form IV (Figure 2, see Figure 4B). Transformation of Form IV to Form III occurred within minutes at room temperature and significantly faster at elevated temperatures, precluding the determination of the melting temperature of Form IV. Crystallization of Form IV from the melt is more preferred in the presence of natural resins such as Canada balsam or gum mastic (ca. 20 wt %), which also slows the transformation to Form III. A reduction in phase transformation rates in the presence of resins and polymers, which has been well established for molecular crystals, can be attributed to slower diffusion and reduced growth of the transformation front.

When the benzamide–resin mixtures are confined between two glass slides, the transformation to Form III can take as long as several days, whereas unconfined benzamide–resin powders transform within hours. Form IV held at T > 70 °C for a few minutes transforms into Form III, even in the presence of resins (Figure 3).

Crystallization under confinement in various nanoporous media often results in changes in polymorph stability rankings and discovery of new polymorphs. The crystallization of benzamide was examined using controlled pore glass (CPG) beads with nominal pore sizes of 4, 8, 35, 50, 100, and 200 nm after infiltration of benzamide melt. PXRD of benzamide confined in CPG beads with pore sizes d ≥ 35 nm revealed 2θ peaks corresponding to the 011, 013, and 102 reflections of Form III (2θ = 16.5, 19.9, and 20.9°, respectively, Figure 4). The diffraction peaks for the benzamide crystals embedded in 8 nm pores, however, differ from those observed in the larger pore sizes, exhibiting unique reflections at 11.4, 18.9, 19.5, and 22.5° that correspond to the new Form IV observed from melt...
crystallization. Numerous reports have demonstrated Debye–Scherrer broadening for reflections corresponding to planes about the zone axis parallel to the pore walls, which can lead to low peak intensities. Moreover, scattering from nanoscale crystals tends to be weak, with broad peaks, which can mask the peaks at 15 and 26° expected from the data for melt crystallization samples. The region near 6° likely indicates the presence of some Form II (admixed with Form IV), which is structurally similar to Form IV. Stronger peaks elsewhere, however, support Form IV as the dominant phase. Under ambient temperature and humidity, confined Form IV has remained stable for more than a year, in contrast to bulk Form IV that transforms to Form III within minutes. Benzamide confined in 4 nm pores does not crystallize even at elevated temperatures below the expected melting temperature for crystals of this size (see below).

The melting temperature of benzamide confined in CPG decreases with decreasing pore size, a direct consequence of the increasing surface-to-volume ratio when crystal size is reduced. The melting point depression can be expressed according to a modified form of the Gibbs–Thomson equation, where $\Delta T_m$ is the melting point depression, $T_m$ is the bulk melting temperature, $T_m(d)$ is the melting temperature of the confined crystal with a diameter $d$ (assumed to be of equivalent size to the host pores), $\Delta H_{fus,bulk}$ is the enthalpy of fusion, $\rho_{solid}$ is the density of the solid, $\gamma_{crystal-melt}$ is the crystal–melt interfacial energy, and $\cos \theta$ is the contact angle between an emerging spherical crystal and the pore wall (or any phase that may have wetted the pore wall).

$$
\Delta T_m = T_m - T_m(d) = -\frac{4T_m\gamma_{crystal-melt}}{d\Delta H_{fus,bulk}\rho_{crystal}} \cos \theta
$$

(eq 1)

The Gibbs–Thomson equation often is simplified by assuming the contact angle $\theta = 180^\circ$ (eq 2).

$$
\frac{T_m - T_m(d)}{T_m} = \frac{4\gamma_{crystal-melt}}{d\Delta H_{fus,bulk}\rho_{crystal}}
$$

(eq 2)

Both eqs 1 and 2 predict a linear relationship between the melting point depression and the inverse of the crystal size. Using the enthalpy of fusion measured for bulk Form III ($\Delta H_{fus,bulk} = 159.4$ J/g) and the density of Form III ($\rho_{crystal} = 1.28$ g/cm$^3$), the slope of $T_m$ vs. $1/d$ gives $\gamma_{crystal-melt} = 40.8(1.1)$ mJ/m$^2$. To evaluate the validity of the interfacial energy obtained, we consider the lower limit of the crystal-melt interface energy, which can be estimated from a semiempirical model.

$$
\gamma_{crystal-melt} = \frac{3}{8N_A} \frac{R\gamma_{crystal-melt}}{\omega^{2/3}} \exp \left( \frac{\Delta H_{fus,bulk}}{3RT_m} \right)
$$

(eq 3)

Here, $R$ is the universal gas constant, $N_A$ is the Avogadro number, and $\omega = 90.4$ cm$^3$/mol is the benzamide molar volume. An estimation of the surface free energy from bulk properties, as in eq 3, gives $\gamma_{crystal-melt} = 50.7$ mJ/m$^2$, which agrees surprisingly well with the experimental values obtained.
Correspondence argues that the assumption of a contact angle $\theta = 180^\circ$ between the confined nanocrystals and the pore walls is reasonable. We note, however, that contact of the confined nanocrystals with an intervening amorphous phase lining the pore walls cannot be discounted. Controlled pore glass beads used in confinement experiments have hydrophilic surfaces and are likely wetted by benzamide, suggesting that the crystals inside pores are separated from the walls by an amorphous benzamide layer. Ultimately, extrapolation of the data for Form III in Figure 5 to 8 nm pore size ($1/d = 0.125$ nm$^{-1}$) indicates a melting point of 88.3 °C. This value is substantially higher than the actual measured value in 8 nm pores, corroborating the crystallization of the less stable Form IV.

**PXRD Data Analysis of Form IV.** While polycrystalline samples of Form IV were obtained from the melt and through nanoscale confinement, single crystals could not be obtained. Moreover, the PXRD data collected on the GADDS micro-diffractometer (Figure 4 and Figure S1) was not sufficient for structure determination. Therefore, structure analysis was performed using synchrotron powder data recorded at the 17-BM beamline ($\lambda = 0.45212$ Å, Advanced Photon Source, Argonne National Laboratory). A two-dimensional pattern collected did not exhibit texture (inset in Figure 6), indicating a polycrystalline sample with no preferred orientation. The powder X-ray diffraction (PXRD) pattern (Figure 6), indexed with the software McMaille v3.04, was consistent with an orthorhombic unit cell ($a = 11.83$ Å, $b = 20.50$ Å, and $c = 5.03$ Å). A Pawley fit of the data in the space group $P2_12_12_1$ afforded $R_{wp} = 5.99\%$. The $hkl$ reflections with $l \neq 0$ were broad and weak, however (Figure S2). A Pawley fit of similar quality ($R_{wp} = 7.1\%$) was obtained using only $hk0$ reflections (with $c$ set to 0.1 Å in the Pawley fitting). Additional high-resolution synchrotron measurements of Form IV were recorded at 11-
Structures Determined by Optimization of the Reported Structures of Forms I, II, and III

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<th>generation method</th>
<th>initial space group</th>
<th>final space group</th>
<th>( E ) (kJ/mol)</th>
<th>( Z' )</th>
<th>( a ) (Å)</th>
<th>( b ) (Å)</th>
<th>( c ) (Å)</th>
<th>( \beta ) (deg)</th>
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*a*Structure generation methods: SA, simulated annealing; CSP, crystal structure prediction. *b*Corresponds to the predicted structure #22 in ref 15. *c*2D lattice parameters in the optimized structure are twice the values of those in SA and CSP structures. *d*With force field, optimized as P1 (Table S3).

BM beamline (\( \lambda = 0.412827 \) Å, Advanced Photon Source, Argonne National Laboratory). Despite higher angular resolution, no additional information was obtained because of significant peak broadening (Figure S3). Similarities in the two measurements imply disorder that is inherent in the benzamide crystal, which allows extraction of only 2D data. Nevertheless, candidate structures for benzamide Form IV can be found using the 2D data.

**Structure Generation.** Pawley fits for all orthorhombic and monoclinic space groups (\( \gamma = 90^\circ \) while \( \alpha \) or \( \beta \) can deviate from 90° due to uncertainty in \( \gamma \) orientation) were evaluated using \( hkl \) reflections (\( c = 0.1 \) Å). The \( P_{21212}, P_{21212}, P_{n2a2}, P_{b1}, P_{n2a}, P_{b1}, P_{mn2}, P_{b1m}, P_{n2m}, P_{b1n} \) space groups provided reasonable fits (\( R_{wp} \leq 8\% \)). These space groups were then used to generate structure solutions by simulated annealing (SA) in Bruker TOPAS 4 software. The geometry of each molecule was defined as a rigid body based on Form I atomic coordinates, and rotational and translational parameters were simultaneously refined during SA search. Due to the severe disorder, only \( hkl \) reflections were used to compare simulated and observed PXRD data. Six structures with the best \( R_{wp} \leq 12\% \) were selected for further computation analysis (SA entries in Table 1 and Table S2). Because simulated annealing did not produce an obvious structure solution, crystal structure prediction (CSP) was employed to generate alternative candidates. Random structures were generated with the UPACK program suite using the general Amber force field (GAFF). Candidate structures were filtered by identifying all matches with CSP-generated unit cells against the indexed parameters of the possible 2D cell for Form IV (within a 5% tolerance; for CSP details, see the Computational Methods section). Five structures of the 34 cells that matched the experimentally determined 2D unit cell parameters (Figure S4) had simulated PXRD patterns with low angle 2\( \theta \) values and intensities consistent with the experimental data for Form IV.

All 11 Form IV candidate structures generated by SA and CSP were ranked by optimizing their structures with density functional theory (DFT). Forms I, II, and III also were optimized for comparison (CSD ref codes: BZAMID02, BZAMID13, and BZAMID12 respectively). Structures of Form IV generated by simulated annealing were first optimized using the GAFF force field embedded in the GULP software with preservation of their respective space group symmetries. The \( P_{b1} \) and \( P_{mn2} \) cells did not reach minimum energy structures unless optimized after the symmetry was reduced to P1. Both the transition state (full symmetry) and local minimum (P1) cells were included as candidate forms. The eight structures from simulated annealing and five structures from CSP were subsequently optimized without space group symmetries using DFT calculations in CP2K v. 2.6.2 (see the Computational Methods section for details). The three structures that optimized to P1 cells with \( Z' = 8 \) are not reported in Table 1 (see Table S3); these include the two saddle point structures from simulated annealing (\( P_{b1} \) and \( P_{mn2} \)) and the \( P_{b1} \) structure optimized as P1 using the classical force field. Optimized structures with unit cells that differed by more than 10% from the experimental 2D lattice parameters were excluded as Form IV candidates. Curiously, three structures optimized to unit cells that resembled the reported \( Fdd2 \) structure of Form III \( P_{b1} \) as well as its optimized version (Table 1), suggesting that Forms II and IV are similar. Consequently, the set of DFT optimized structures (Table 1, Figure 7) was narrowed to four potential candidates for Form IV: \( Fdd2 \) (4.48 kJ/mol), \( P_{21212} \) (4.68 kJ/mol), \( P_{21212} \) (7.44 kJ/mol), and \( P_{21212} \) (8.21 kJ/mol).

**Description of Candidate Structures.** The four aforementioned candidate structures adopt a parquet-like motif of tiles, each consisting of a tetrad of benzamide molecules, stacked normal to the 5 Å axis. The tiles are parallellograms with nearly the same dimensions; the length of one side approximately twice the length of the other, \( l = 2w \), and an angle \( \phi \) between the two sides (Figure 8). The tetrads in the candidate Form IV structures resemble those in Form II, but the tiles have different dimensions (\( w = 7.8 \) Å and \( \phi = 82^\circ \) in Form IV vs \( w = 8.3 \) Å and \( \phi = 63^\circ \) in Form II). Unlike Forms I and III, in which benzamide molecules are associated by hydrogen-bonded \( R_2(8) \) dimers that assemble further through dimeric hydrogen-bonded \( R_2(8) \) tapes, Form IV forms skewed \( R_2(8) \) rings from three benzamide molecules that assemble into a hydrogen-bonded \( R_2(8) \) catemeric tape (Figure 9). Candidate structures with the \( R_2(8) \) dimers observed in Forms I and III resulted in optimized unit cells that did not correspond with the PXRD cell of experimental
Form IV (Table S4). One of the P1 structures (Table S4, 7.90 kJ/mol) does contain $R_2^2(8)$ dimers but is unlikely because $Z' = 8$.

Figure 7. Four candidate Form IV structures. All four structures consist of benzamide tetrad tiles that stack along the short 5 Å axis (normal to the plane of the page) through catemeric hydrogen-bonds. With the exception of $P2_{1}/n$ structure, all the carbonyls within a tile point in the same direction. The energy values for each structure are relative to optimized Form I (Table 1).

Figure 8. Molecular packing of the tetrads in the four Form IV candidate structures.

Figure 9. Hydrogen-bonding motifs for Form IV candidate structures (left) and Forms I and III (right). Graph set notations are indicated.

All four candidates of Form IV contain three types of phenyl—phenyl contacts within the tetrads and between tetrads. T- and E-contacts describe interactions between neighboring tiles (Figure 8). T-contacts, in which a (phenyl) C—H bond points toward a phenyl ring on a neighboring tetrad, occur in all candidate structures and are characterized by dihedral angles of $68°−78°$. External contacts, E, between parallel tiles associate in two motifs. V-contacts (for the V-shaped geometry of the two neighboring rings) adopt an edge-to-face herringbone arrangement, with dihedral angles $E_V = 67°−72°$ between neighboring phenyl rings. P-contacts (for the parallel arrangement of two neighboring rings) exhibit dihedral angles $E_p = 0°−7°$. I-contacts describe phenyl ring associations within a tile (Figure 8). $Fdd2$ and $P2_{1}2_{1}2$ belong to one family with internal V-contacts, characterized by
dihedral angles of $I_p = 67°$ to $69°$. In contrast, $P_{21}2_12_1$ and $P_{21}/n$ form a second family of structures, all with nearly parallel phenyl rings (P-contact) with corresponding dihedral angles $I_p = 10°$ to $11°$ (Table S5).

The two families of candidates were evaluated by Rietveld refinement using the DFT-determined structures of Form IV. Although the $R_{wp}$ values of all four structures were high, the structures with the $I_p$-contacts ($Fdd2$, $P_{21}2_12_1$; Figures 7, 8) exhibited the best fits ($R_{wp}$) to the experimental data, suggesting this type of tile is more likely than $I_p$-contacts in pointing in enantiopolar directions, is distinguished as $u$ subscripts $p$ sizes and symmetries. One particularly interesting example that effectively combines the tiling pattern of $Fdd2$ and $P_{21}2_12_1$ is a low energy (8.30 kJ/mol relative to Form I) orthorhombic $Pca2_1$ structure with $Z = 4$ and $I_p$-contacts as well (Figure S6). This structure results in different domain interfaces.

The energies of the interfaces between the nanoscale domains were calculated by molecular dynamic (MD) simulations that combine two enantiomorph or enantiopolar building blocks. The corresponding energies of the bulk structures (see the Experimental Section and Figure 11) were compared. The calculated interface energies were comparable (Table 3) to the surface energies determined for crystal—melt interface energies of ca. 41 mJ/m² obtained from the DSC data on crystallization in confinement (see above).

**Disorder Model.** Distinct peaks associated with $hkl$ reflections for $l ≠ 0$ are absent in the experimental PXRD pattern. The $hk1$, $hk2$, and $hk3$ Bragg reflections, expected at $2θ < 18°$, were effectively extinct, which can be a signature of a 2D-stacking fault disorder. Using atomistic models, it is possible to simulate the disorder among neighboring stacks of tiles and the associated PXRD patterns. The $Fdd2$ structure consists of tiles in which the all carbonyl groups orient along the same direction, at 0, 1/4, 1/2, 3/4, parallel to the 5 Å tape axis. The $P_{21}2_12_1$ structure consists of the same tiles but with one-half of the carbonyl groups oriented up, at 0, 1/2, and the other half down, at 0, 1/2, with respect to the tape axis. Disorder models, each consisting of $20 \times 20 \times 1$ supercell, were built from random combinations of single stacks corresponding to the $Fdd2$ and $P_{21}2_12_1$ structures and PXRD pattern generated using ZMC and DISCUS software suites (see the Computational Methods section for details) and then compared to the experimental pattern. It is important to note that a random collection of $Fdd2$-like all “up” and all “down” tapes (where up and down denote the direction of the carbonyl groups), combined with translations of 0, 1/4, 1/2, 3/4 along the tape axis, is indistinguishable from a random arrangement of $P_{21}2_12_1$-like tapes. Three possible settings were used to characterize the disorder using the $Fdd2$-like tapes only: random tape orientation (C1), random tape displacements (in increments of 1/4) along the short axis (C2), and both (C3). Figure 10 depicts the random distribution of orientations (random tape displacements cannot be discerned from this figure). This kind of analysis is commonly used for the interpretation of single crystal X-ray diffuse scattering or pair distribution functions.

![Figure 10. Idealized tiling motifs for internal V-contact structures $Fdd2$ and $P_{21}2_12_1$ (with enantiomorphs), $Pca2_1$, and an example of a disordered structure. Unit cells are outlined in blue. Dark and light green tiles represent tetrads of benzamide molecules in which all the C=O vectors are pointing out of and into the page, respectively. These schemes do not distinguish the relative shifts of catemers normal to the page.](https://doi.org/10.1021/acs.cgd.0c00096)

Although the choices for the candidate structures were narrowed to $Fdd2$ and $P_{21}2_12_1$, both with $I_p$ contacts, disorder models were also built from the tiling in the $P_{21}2_12_1$ and $P_{21}/n$ structures, which have $I_p$ contacts to confirm that the former pair represented the best candidate structures. Pattern B in Figure 12 corresponds to the $I_p$ model with both random orientations and displacements. Comparison with the Form IV experimental data (pattern A) shows significant disagreements in peak intensities. The diffuse scattering calculated at $2θ ~ 5°$ for $I_p$ models is not apparent in the experimental data, thus arguing against the two $I_p$ candidates ($P_{21}2_12_1$, $P_{21}/n$; Figure 7).

Inspection of the PXRD patterns (Figure 12) calculated for the aforementioned disordered $I_p$ models C1, C2, and C3 reveals distinct differences. The random tape orientations in the C1 model do not distinguish $hkl$ reflections adequately for $l ≠ 0$, as evidenced by peaks in the C1 pattern that are not observed in the experimental data (e.g. peaks at $2θ = 6.1, 6.5, 8.4°$). C2, with random tape displacements, and C3, with both...
random tape displacements and orientations, extinguish the $hk_1$, $hk_2$, and $hk_3$ Bragg reflections, similar to the experimental PXRD data. The extinction of $hk_1$, $hk_2$, and $hk_3$ reflections also can be validated from the corresponding reciprocal space sections for $C1$ and $C3$ calculated using DIFFUSE.\(^7\) (Figure 12). Collectively, these observations demonstrate that disorder of the tape orientations alone is not sufficient to explain the observed data.

Crystal Adiabatic Free Energy Dynamics (C-AFED) Analysis. The PXRD data is consistent with the presence of 2D disorder in Form IV, explained further through modeling as related to displacement of the tapes along the short stacking axes. This does not imply that a mechanical crystal distortion allows tiles to slip past each other, as faults also could occur during crystal growth. Alternatively, disorder could reflect nearly equal energies for the possible domain boundaries between stacks of tiles.\(^7\) One approach for testing the propensity of a structure to exhibit stacking faults is to simulate deformations using molecular dynamics (MD).\(^7\) In particular, C-AFED simulations can accommodate unit cell fluctuations that might lead to disorder.\(^3\),\(^4\)

Comparison of initial (after NPT_F equilibration) and final frames from 500 ps C-AFED simulations for $Fdd2$ and $P2_1212$ are shown in Figure 13. The blue and red colors represent the magnitude of atomic $z$-displacements from the base of the simulation box. The $Fdd2$ molecules appear relatively unchanged in their positions between the first and final frames. In contrast, the $P2_1212$ simulation shows clear fluctuations of the molecules along the $z$-displacement, as evidenced by the skewed stripes of color for molecules that started with the same $z$-displacement. This suggests that the $P2_1212$ tiling can accommodate displacements more readily than the $Fdd2$ form. These stacking faults are consistent with the disorder models that best match the PXRD data. While the $Fdd2$ tiling is lower in energy and more structurally stable in the MD simulations, the lack of characteristic peaks in the PXRD data rules out domains larger than just a few tetrad tiles. Combining the SRO models with the MD results, we find that Form IV likely incorporates small $Fdd2$-type domains with varied orientations relative to their nearest neighbors, as shown by the disordered structure in Figure 10.

■ CONCLUSIONS

A new, highly metastable benzamide Form IV polymorph was discovered by melt crystallization in parallel with growth under nanocofiguration in very small pores. Under nanoscale confinement, the melting behavior of more stable benzamide
Fluctuations in the positions relative, periodic positions of the molecules in the initial frames on the z-axis are evidence of stacking faults. The insets correspond to xy sections viewed along the z-axis for each frame.

Form III crystallizing in larger pores was strongly influenced by the pore size, with the melting depression scaling inversely with crystal size. Interfacial energies calculated by the Gibbs–Thomson equation agreed with those obtained by semi-empirical model. Using a combination of powder X-ray diffraction analysis and computational methods, a large number of candidate structures for benzamide Form IV were reduced to a few possible disorder models based on tapes of 2D parquet-like tiles consisting of benzamide tetrad. The disorder could be explained by various stacking fault configurations corresponding to random tape displacements and orientations. Collectively, these observations reveal an impressive crystallographic complexity for such a simple molecule, yet one that is within reach of emerging methods for determination of solid-state structure. Moreover, this analysis suggests that many structures that may have been abandoned because of catch-all shortcomings, such as poor crystallinity, twinning, or disorder, can be solved.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.cgd.0c00096.

Tables of CPG pore manufacturer specifications, space groups selected, DFT-optimized unit cell parameters and relative lattice energies, hydrogen bonding and phenyl arrangement motifs, and structural parameters for candidate structures and figures of PXRD patterns, 17-BM beamline synchrotron diffraction data, comparison of synchrotron powder diffraction patterns, energy vs molecular volume plot, overlay of calculated PXRD patterns, and projections of structures (PDF)

Five hundred lowest energy structure from CSP (CIF) Thirty-five 2D cells from the initial CSP search consistent with the experimental lattice parameters for hk0 indices (CIF) Seventeen 3D structures CSP and simulating annealing corresponding to entries in Table 1 and Table S2 (CIF)

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**Notes**

The authors declare no competing financial interest.

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molecule reveals its secrets the crystal structure of the unstable Pulham, C. R.; Shankland, K. Woehler and Liebig revisited: A small assistance in collecting synchrotron powder diffraction data at Advanced Photon Source. The authors thank Prof. Qiang Zhu for a preliminary crystal structure prediction search and Prof. Martin Schmidt for helpful discussions on benzamide structure.

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