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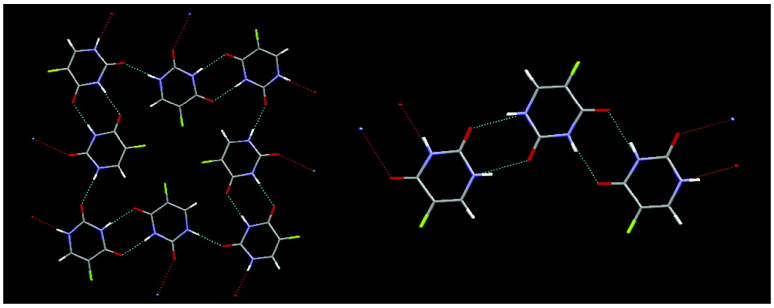

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A New Polymorph of 5-Fluorouracil Found Following Computational Crystal Structure Predictions

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5-Fluorouracil was first synthesized in 1957,[1] and in the intervening 45 years it has been used extensively in the treatment of solid tumors.[2] Only one crystal structure is reported in the literature for pure 5-fluorouracil,[3] hereafter referred to as Form I. In this structure, the compound crystallizes with four molecules in the asymmetric unit in the space group $P\bar{1}$. The molecules adopt a hydrogen-bonded sheet structure. Regions occur within each sheet where four fluorine atoms are in close proximity, approaching within 3.2 Å. This unusual feature and the complexity of the crystal structure required investigation. A computational crystal structure search was performed and generated a range of hypothetical crystal structures at lower lattice energies than the known structure. A manual crystallization screen, performed concurrently to obtain crystals from a range of crystallization environments, yielded a new polymorph (Form II) that corresponded to the global energy minimum structure from the computational search.

Initial hypothetical crystal structure generation was performed by MOLPAK,[4] which systematically generates densely packed structures in the common packing types for organic molecules (limited to 17 space groups, $Z' = 1$), which precludes finding Form I in the search. After generation of the initial crystal structures, using the MP2 6-31G** ab initio optimized molecular structure, lattice energy minimization was performed using DMAREL[5] to move these structures to an energy minimum on the packing energy hypersurface. The lattice energy was calculated using an exp-6 dispersion−repulsion model with the FIT potential parameters[6] supplemented by F parameters[7] and the electrostatic contribution from a distributed multipole analysis (DMA) of the MP2 6-31G** charge density of the molecule, with multipoles up to hexadecapole centered on all atomic sites. This ab initio based molecular structure and intermolecular potential reproduced the crystal structure of Form I satisfactorily, with the greatest lattice parameter variation between the experimental structure and the energy minimized structure being 3.7%. The search produced more than 200 distinct hypothetical structures within 20 kJ mol^{-1} of the global energy minimum structure (Figure 1).

The manual crystallization screen used a range of 18 solvents in which 5-fluorouracil was soluble and employed six crystallization techniques. These techniques were solvent evaporation, vapor diffusion with diethyl ether acting as anti-solvent, vapor diffusion with toluene acting as anti-solvent, reverse vapor diffusion with toluene acting as anti-solvent, solvent evaporation at 5 °C (in a domestic refrigerator), and cooling to 5, −10, or −40 °C, depending on the level of cooling required to initiate precipitation.

All suitable crystals produced from the crystallization screen were investigated using single-crystal X-ray diffraction, with full data sets collected and structures determined for any new crystalline modifications. During the course of the study, the crystal structure of Form I was redetermined at low temperature (150 K), with all

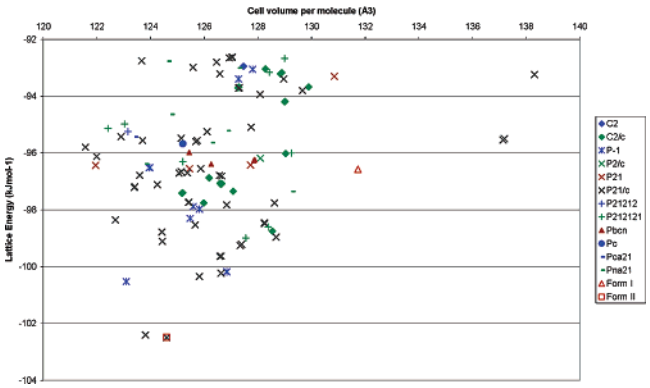

Figure 1. Scatter plot showing the cell volume per molecule against the lattice energy for all hypothetical structures within 10 kJ mol^{-1} of the global energy minimum. The lattice energy minima corresponding to the experimental crystal structures are also shown.

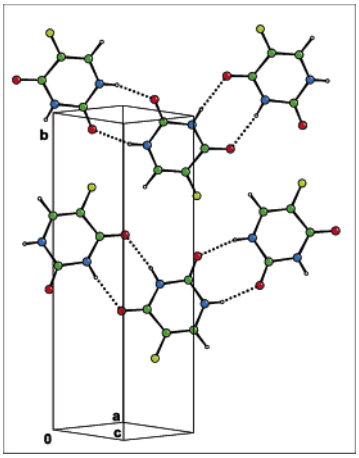

Figure 2. Hydrogen-bonding motif present in Form II of 5-fluorouracil and the lowest energy hypothetical structure. Two pairs of hydrogen bonds connect each molecule in the ribbon to its neighbors. Diagram produced using CAMERON.[9]

atomic positions (including hydrogen atoms) located from the electron density map. Three new crystal structures were discovered that contained both 5-fluorouracil and a single solvent species within the crystal lattice. These three solvate structures contained dimethyl sulfoxide, dimethyl formamide, and 1,4-dioxane. The structures of these solvates have been reported elsewhere.[8]

The newly discovered polymorph of 5-fluorouracil is denoted Form II and crystallizes in the space group $P2_1/c$ with a single molecule in the asymmetric unit. This structure contains a hydrogen-bonded ribbon motif, with each molecule forming two dimer hydrogen bond pairs to its neighboring molecules (Figure 2). The ribbons lie parallel to one another to form rippled layers. No specific

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interactions occur between ribbons in each layer. The layers stack as the (−1 0 2) Miller planes to form the three-dimensional crystal structure.

Two hydrogen-bonding motifs commonly recur in the subset of the 61 unique lowest energy distinct hypothetical structures within 8 kJ mol^{-1}, which differ from the motif in Form I in that each molecule is hydrogen-bonded to two nearest neighbors rather than three.

In four of the five lowest energy structures, there are two hydrogen-bonded dimer pairs per molecule as shown in Figure 2. The most common motif, present in 24 of the structures, is a ribbon closely related to the motif seen in several analogues of 5-fluorouracil, namely 5-chlorouracil[10] and 5-bromouracil,[10] which are isostructural, and thymine.[11] In this motif, each molecule forms two hydrogen-bonded dimer pairs, which share a common carbonyl hydrogen bond acceptor.

When the single-crystal X-ray structure of Form II was obtained, it corresponded to the predicted structure at the global minimum in the lattice energy. This suggests that Form II is the thermodynamically stable form, at least at low temperature, as the calculations correspond to 0 K structures. Thermal analysis, however, suggests a monotropic relationship with Form I being more stable, as discussed in the Supporting Information.

The crystallization method that yielded Form II was solvent evaporation of a saturated solution of the compound in nitromethane at room temperature over a period of three months. Further samples of Form II have been prepared but only from dry nitromethane. The vast majority of crystallizations of 5-fluorouracil give either Form I or a solvate. The facile formation of Form I, the prevalence of solvate structures, and the specific conditions required to produce Form II, indicate that 5-fluorouracil does not have a well-defined low energy crystallization pathway that leads to a single energetically stable structure. In nonsolvate-forming solvents, the crystallization pathway to Form I is favored.

A kinetically reasoned hypothesis can account for the different crystallization results from water (Form I) and dry nitromethane (Form II), which could be extended to explain the crystallization results from other solvents. In the presence of water, the C═O and N−H functional groups are tightly solvated by water molecules, mitigating against the formation of the doubly hydrogen-bonded dimers that would be expected in the gas phase. The fluorine atoms are much less strongly solvated by water and allow the 5-fluorouracil molecules to associate through F···F contacts. The regions in the Form I sheets with F···F close contacts would be consistent with this postulation. Previous work[12] has shown that F···F interactions can be influential in directing the crystal structure adopted by fluorine-containing organic molecules. Form II crystallizing from dry nitromethane has no close F···F contacts, but also no water content to hinder the formation of the hydrogen-bonded dimers. 5-Fluorouracil crystallizes from wet nitromethane as Form I. Crude calculations taking into account the hydroscopicity of nitromethane suggest a wet solution would have between 4 and 40 water molecules to each fluorouracil molecule. This would be sufficient to form a hydration sphere round the C═O and N−H functional groups, supporting the hypothesis. Ongoing molecular dynamics studies[13] on 5-fluorouracil in water are consistent with this hypothesis.

It is notable that a new polymorph of a commonly used pharmaceutical has only been found after 30 years from a search inspired by computational predictions. In this case, the calculations have not only predicted a new hydrogen-bonding motif,[14] but also the unit cell within a few percent error. The predicted stability of the new form is consistent with its hydrogen-bonding motif, and the notable difficulty in crystallizing this structure is consistent with the differential solvation of the functional groups within the molecule. Given that the predictions suggest that structures with another hydrogen-bonding motif could also be stable, we are also attempting to crystallize such a form by templating with molecules that crystallize with this motif, in the hope that this will facilitate the required disruption of the solvation structure and the realization of a third polymorph.

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Supporting Information Available: CIF files of the redetermination of Form I (150 K) and Form II (room temperature and 150 K) of 5-fluorouracil. Details of all crystallization experiments. DSC data and traces and a discussion of the relative thermodynamic stability of the two forms including an analysis of the computational model. Details of the structures within 8 kJ mol^{-1} of the global minimum from the hypothetical crystal structure prediction search, giving reduced cell parameters, space groups, energy ranking, and hydrogen bond analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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