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Experimental and Computational Hydrate Screening: Cytosine, 5-Flucytosine and Their Solid Solution

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Abstract

The structural, temperature- and moisture dependent stability features of cytosine and 5-flucytosine monohydrates, two pharmaceutically important compounds, were rationalized using complementary experimental and computational approaches. Moisture sorption/desorption, water activity, thermal analysis and calorimetry were applied to determine the stability ranges of hydrate ↔ anhydrate systems, while X-ray diffraction, IR spectroscopy and crystal structure prediction provided the molecular level understanding. At 25 °C, the critical water activity for the cytosine hydrate ↔ anhydrate system is ~0.43 and for 5-flucytosine ~0.41. In 5-flucytosine the water molecules are arranged in open channels, therefore the kinetic desorption data, dehydration < 40% relative humidity (RH), conform with the thermodynamic data, whereas for the cytosine isolated site hydrate dehydration was observed at RH < 15%. Peritectic dissociation temperatures of the hydrates were measured to be 97 °C and 84.2 °C for cytosine and 5-flucytosine, respectively, and the monohydrate to anhydrate transition enthalpies to be around 10 kJ mol⁻¹. Computed crystal energy landscapes not only revealed that the substitution of C5 (H or F) controls the packing and properties of cytosine/5-flucytosine solid forms, but also have enabled the finding of a monohydrate solid solution of the two substances which shows increased thermal- and moisture-dependent stability compared to 5-flucytosine monohydrate.

Keywords

hydrate; solid solution; crystal structure prediction; calorimetry; moisture sorption/desorption

1 Introduction

Most drug compounds are administered as solid oral dosage forms, with tablets being the most popular. The dominance of solid drug formulations is because the chemical stability of a molecule is usually much higher in the solid state than in solution. Therefore, for instance, also injectable drugs are often formulated as powders and dissolved or dispersed in a sterile liquid right before application. However, even if a drug compound is formulated as solution the solid state of the substance may be of great importance for purification or in certain

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stages of the manufacturing process.¹ Thus, a thorough knowledge of the solid forms of the drug substance and their properties is essential in the modern drug development and for solid formulations, the identification of the most appropriate solid state form is a critical step in the development of high-quality (drug) products. The experimental solid form landscape can encompass single and multi-component forms (hydrates, solvates, salts, cocrystals, solid solutions, etc.).

Polymorphism, a well-known solid state phenomenon, can occur for single as well as multi-component systems and implies that a compound crystallizes with more than one distinct crystal structure but identical chemical composition.² Solvate formation occurs when the solvent of crystallization becomes part of the crystal lattice,³ with the largest number of solvates containing water (hydrates).^{4–6} Solvates (hydrates) often crystallize more easily than the neat forms because the inclusion of the solvent molecule may lead to stronger intermolecular interactions between the host and guest (solvent) molecules and to a more efficient packing. Solvate, and in particular hydrate formation, may not be avoidable and has many implications in the fine chemical industries, because it affects similar to polymorphs the physicochemical properties of materials, such as solubility, dissolution rate, density, etc. and thus, can influence the manufacturability, stability and efficiency of products.⁷ Phase transitions, such as polymorph interconversion,^{8,9} desolvation,^{10–12} hydrate formation,^{13,14} and crystalline conversion,^{15,16} may occur during various processes. In a solid solution the solute (e.g., an organic compound) may incorporate into the crystal lattice substitutionally, by replacing a molecule in the lattice, or interstitially, by filling structural voids in the crystal structure. Both types affect the properties of the material by distorting the crystal lattice.

Understanding the hydration/dehydration of organic solids has a vast scientific background comprising a broad range of thermodynamic, kinetic and structural understanding.^{17–19} Numerous classifications have been proposed. For example, Morris and Rodriguez-Hornedo^{3,20} introduced a structural classification system. *Isolated site hydrates*, retain water molecules in segregated pockets in the crystal structure. Removal of the water molecules requires an appreciable driving force ensuring a disruption of the hydrate crystal structure. In *channel hydrates*, the water molecules are located in tunnels or connected pockets. The water molecule may be (highly) mobile and water egress/ingress may occur readily with moderate change in temperature or relative humidity (RH). In metal *ion-associated hydrates* the water molecules are bound directly to an ion.

The experimental form screening is routinely conducted in fine chemical industries to ensure that all forms have been found and that the most appropriate solid form is developed.^{21,22} Characterization of the resulting forms may be complicated by the fact that non-stoichiometric (content is variable within a certain range), mixed solvates or solid solutions are formed. Herein we demonstrate that the combination of appropriate experiments and computational modelling may overcome critical hurdles in characterizing solid forms emerging from a hydrate screening programme using cytosine (6-amino-2(1*H*)-pyrimidinone, Figure 1a) and 5-flucytosine (6-amino-5-fluoro-2(1*H*)-pyrimidinone, Figure 1b).

Cytosine is one of the pyrimidines found in the deoxyribonucleic acid. The derivative 5-flucytosine has been used for the treatment of fungal infections since 1968^{23,24} and is on the World Health Organization's List of Essential Medicines. A more recent indication is its use as a prodrug in tumour cell treatment.²⁵ Both, cytosine and 5-flucytosine, exhibit polymorphism. The Cambridge Structural Database (CSD)²⁶ contains each two distinct anhydrate structures for cytosine (CYTSIN27 and CYTSIN0128 correspond to one polymorph: **C-I**; CYTSIN0229: **C-II**) and 5-flucytosine (MEBQEQ0130: **F-I**; MEBQEQ30: **F-II**). One monohydrate form is known for cytosine (Refcode family: CYTOSM^{28,31–35}). On the other hand, 5-flucytosine shows a rich hydrate solid form landscape, one hemihydrate (DUKWIQ36), two monohydrates (BIRMEU37, BIRMEU0138, BIRMEU0230: **fH1-I**; BIRMEU0330: **fH1-II**) and one hemipentahydrate (MEBQUG30). Solvate formation has only been reported for 5-flucytosine (MEBQOA30: methanol, MEBQIU30: 2,2,2-trifluoroethanol, DUKWEM36: dimethyl acetamide and DUKWAI36: dimethyl sulfoxide). The two investigated compounds differ solely in the substitution of the C5 position, either H for cytosine and F for 5-flucytosine. Neither of the two atoms is part of a strong hydrogen-bonding donor or acceptor functional group, which may imply that similarities in the solid state are observed, e.g. isostructures, as seen for other compounds differing only in the substitution of H- and F-atoms (e.g. 4,5'-substituted benzenesulfonamido-2-pyridines³⁹).

Despite being important substances hardly any information on solid form stability and interrelations of the forms can be found in the literature. Therefore, this present work seeks to establish the thermodynamically stable neat and hydrated cytosine and 5-flucytosine solid forms at ambient conditions and to unravel the structural similarities and dissimilarities of the two compounds' solid state forms. A broad range of analytical techniques were applied, including hot-stage microscopy (HSM), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), relative humidity (RH)-perfusion calorimetry, X-ray diffractometry (powder and single crystal), Infrared spectroscopy (IR), gravimetric moisture sorption/desorption analysis and water activity measurements (slurry method). The experimental findings were complemented with the computational generation of the cytosine and 5-flucytosine monohydrate crystal energy landscapes, which suggested the existence of either cytosine monohydrate polymorph(s) or a monohydrate co-crystal of the two compounds or a solid solution thereof. The presence of a solid solution could be verified with computational (lattice energy modelling) and experimental data, incl. its single crystal structure. Hence, we provide a complete temperature- and moisture-depending stability picture of the stable hydrate and anhydrate forms, solid solutions of cytosine and 5-flucytosine and present another successful application of crystal structure prediction (CSP)^{40,41} to complement and interpret experimental findings⁴² in solid state chemistry.

2 Materials and Methods

2.1 Materials and Experimental Solid Form Screening

A commercial cytosine sample, purchased from Sigma (Lot# SLBF0582V, purity 99%), and an in-house 5-flucytosine sample (Ro 2-9915, purity 99%) were used for the

investigations. The cytosine sample consisted of **C-I** and the 5-flucytosine sample of a mixture of **F-I** and **F-II**. The solvents used for the experiments were all of analytical grade.

The experimental solid form screen encompassed sublimation, slurry experiments in water or aqueous solvents, systematic dehydration studies, systematic seeding experiments, low-temperature differential scanning calorimetry (DSC) experiments and solvent evaporation experiments.

Sublimation experiments were carried out on a Kofler hot bench in the temperature range between 230 and 270 °C. The samples were prepared between two glass slides separated by a spacer ring of 5–10 mm height. This method yielded two distinct polymorphs for cytosine, **C-I** and **C-II**, and one anhydrate for 5-flucytosine (**F-I**).

Cytosine **C-I** and a mixture of **F-I** and **F-II** were each subjected to numerous slurry experiments: (i) in water:methanol mixtures at 25 °C (see section 1.3 of the Supporting Information), (ii) in water covering the temperature range from 2 to 65 °C (see section 1.5 of the Supporting Information) and (iii) in n-butanol covering the temperature range from 10 to 30 °C (see section 1.6 of the Supporting Information), (iv) for cytosine 30 different organic solvents were chosen (section 1.13.1 of the Supporting Information) and the temperature range from 10 to 20 °C was covered.

Systematic dehydration studies were performed for **cH1** and **fH1-I** varying the dehydration temperature and RH (see section 1.9 of the Supporting Information). Low-temperature DSC experiments were performed for the anhydrides (**C-I**, **C-II**, **F-I** and **F-II**) and monohydrates (**cH1** and **fH1-I**) covering the temperature range from 20 to –120 °C (see section 1.6 of the Supporting Information).

Solvent evaporations from mixtures of methanol with organic solvents were performed for cytosine (section 1.13.2 of the Supporting Information). Additional solvent slurry, crystallization and evaporation experiments were performed for the two substances from tetrahydrofuran (THF) and a THF/water mixture (see section 1.7 of the Supporting Information), resulting in the known experimental solid forms.

2.2 Gravimetric Moisture Sorption/Desorption Experiments and Determination of the Critical Water Activity (Slurry Method)

Moisture sorption and desorption studies were performed with the automatic multisample gravimetric moisture sorption analyzer SPS23-20 μ (ProUmid, Ulm, D). The moisture sorption analyzer was calibrated with saturated salt solutions according to the supplier's recommendations. Approximately 110 - 130 mg of sample was used for each analysis. The measurement cycles were started at 40% RH (relative humidity) with an initial stepwise sorption (increasing humidity) to 95 %, followed by a desorption cycle (decreasing humidity) to 0% RH and a final sorption step to 95% RH. RH changes were set to 5% for all sorption/desorption steps. The equilibria conditions for each step were set to a mass constancy of ± 0.001 % over 60 minutes and a maximum time limit of 48 hours.

Excess of **C-I** and a mixture of **F-I** and **F-II** polymorphs were stirred separately (~500 r.p.m.) in 0.5 mL of methanol/water mixtures, each containing a different mole fraction of water corresponding to a defined water activity^{43,44} (Section 1.3 of the Supporting Information) at 25.0 ± 0.1 °C for 10 (cytosine) or 21 (5-flucytosine) days. Coulometric Karl Fischer Titration (C20 instrument, Mettler Toledo, CH) was used to determine the water content in the solvent mixtures. Samples were withdrawn periodically and the resulting phase (wet cake) was determined using PXRD.

2.3 Thermal Analysis (TA) and Isothermal Calorimetry (IC)

A Reichert Thermovar polarization microscope, equipped with a Kofler hot-stage (Reichert, A), was used for *hot-stage thermal microscopy (HSM)* investigations. Photographs were taken with an Olympus DP71 digital camera (Olympus, D).

Differential Scanning Calorimetry (DSC) thermograms were recorded either with a DSC 7 or Diamond DSC (Perkin-Elmer Norwalk, Ct., USA) and controlled by the Pyris 7.0 software. Using a UM3 ultramicrobalance (Mettler, Greifensee, CH), samples of approximately 1 – 17 mg were weighed into perforated or sealed aluminium pans or high-pressure capsules. The samples were heated using rates in between 1 and 50 °C min⁻¹ with dry nitrogen as the purge gas (purge: 20 mL min⁻¹). The two instruments were calibrated for temperature with pure benzophenone (mp 48.0 °C) and caffeine (236.2 °C), and the energy calibration was performed with indium (mp 156.6 °C, heat of fusion 28.45 J g⁻¹). The errors on the stated temperatures (extrapolated onset temperatures) and enthalpy values were calculated at the 95% confidence intervals (CI) and are based on at least five measurements.

Thermogravimetric Analysis (TGA) was carried out with a TGA7 system (Perkin-Elmer, Norwalk, CT, USA) using the Pyris 2.0 Software. Approximately 3 – 8 mg of sample was weighed into a platinum pan. Two-point calibration of the temperature was performed with ferromagnetic materials (Alumel and Ni, Curie-point standards, Perkin-Elmer). Heating rates of 2 – 10 °C min⁻¹ were applied and dry nitrogen was used as a purge gas (sample purge: 20 mL min⁻¹, balance purge: 40 mL min⁻¹).

Isothermal calorimetry (IC) experiments were performed with the TAM III nanocalorimeter unit (TA Instruments, Eschborn, D) in a 4 mL stainless steel RH-perfusion ampoule. The RH was controlled with two mass flow controllers and dry N₂ was used as carrier gas at a constant flow rate of 100 mL h⁻¹. Approximately 25 mg cytosine and 18 – 20 mg 5-flucytosine was used. For cytosine the humidity profile (% RH vs. time) was executed as follows: 80% to 0% RH in one step (dehydration: **chI** to **C-I**) and 0% to 80% in one step (hydration: **C-I** to **chI**). For 5-flucytosine the hydration reaction of **F-I** and **F-II** to **fhI-I** was executed as follows: 40% RH to 95% RH in one step. The RH perfusion cell was calibrated with saturated solutions of NaCl (75.3% RH), Mg(NO₃)₂ (52.8% RH) and LiCl (11.3% RH). The heat flow of the empty RH perfusion ampoule (baseline runs with the same humidity steps) was subtracted from the heat flow of the sample measurements. The errors on the stated (de)hydration enthalpy values are calculated at the 95% confidence intervals (CI) and are based on three measurements.

2.4 Powder and Single Crystal X-Ray Diffraction

Powder X-ray diffraction (PXRD) patterns were obtained using an X'Pert PRO diffractometer (PANalytical, Almelo, NL) equipped with a θ/θ coupled goniometer in transmission geometry, programmable XYZ stage with well plate holder, Cu-K $_{\alpha 1,2}$ radiation source with a focussing mirror and a solid state PIXcel detector. The patterns were recorded at a tube voltage of 40 kV and tube current of 40 mA, applying a step size of $2\theta = 0.013^\circ$ with 80 s or 200 s per step in the 2θ range between 2° and 40° . For non-ambient RH measurements, a VGI stage (VGI 2000M, Middlesex, UK) was used. The PXRD patterns, recorded at 25°C , were indexed using the first twenty peaks with DICVOL04 and the space group was determined based on a statistical assessment of systematic absences,⁴⁵ as implemented in the DASH structure solution package,⁴⁶ and agreed with the single crystal data allowing for temperature effects. Pawley fits⁴⁷ were performed with Topas Academic V5.⁴⁸ The background was modelled with Chebyshev polynomials and the modified Thompson-Cox-Hastings pseudo-Voigt function was used for peak shape fitting.

Single Crystal X-ray diffraction (SCXRD) experiments. Single crystals of a monohydrate solid solution of cytosine and 5-fluorocytosine were obtained from cooling crystallization experiments of 16.0 mg substance (equimolar ratio of cytosine and 5-fluorocytosine) from 1 mL of water. Crystallization occurred at $\sim 25^\circ\text{C}$. The data set (Mo radiation; $\lambda = 0.7107 \text{ \AA}$) was collected on an Oxford Diffraction Gemini-R Ultra diffractometer operated by CrysAlis software.⁴⁸ The structure was solved by direct methods (SIR201150) and refined by full-matrix least squares on F^2 using SHELXL201351 and the program package WinGX.⁵² All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were located in difference maps. The hydrogen atoms bonded to C5 atoms ($Z' = 2$) were refined with a distance restraint of $\text{C-H} = 0.95(2) \text{ \AA}$, with U_{iso} parameters set at $1.2 U_{\text{eq}}$ of the parent C atom. Either a F- or H-atom is connected to the C5 position in the solid solution. The partial occupancies refined as follows. Molecule 1: 47% F and 53% H; molecule 2: 50% F and 50% H. For details see ref 49.

2.5 Infrared spectroscopy

Infrared spectra were recorded with a diamond ATR (PIKE GaldiATR) crystal on a Bruker Vertex 70 spectrometer (Bruker Analytische Messtechnik GmbH, D). The spectra were recorded in the range of 4000 to 400 cm^{-1} at an instrument resolution of 2 cm^{-1} (32 scans per spectrum).

2.6 Theoretical Calculations

Cytosine and 5-fluorocytosine are known to exist (at least in solution) in different tautomeric forms. A survey of the two compounds' structures present in the Cambridge Structural Database²⁶ revealed that so far only the keto (amino-keto) tautomer has been identified in solid state (see section 1.1 of the Electronic Supporting Information). Therefore, only the keto tautomer was considered in our computational search for monohydrate structures. The molecular conformation was *ab initio* optimized at the PBE0/6-31G(d,p) level using Gaussian0950 and this optimized conformation was used as input for the computational generation of monohydrate structures.

The hypothetical crystal structures were generated with the program CrystalPredictor.^{51–53} Each 500000 cytosine and 500000 5-flucytosine monohydrate structures were randomly generated in 48 space groups (section 2.1.2 of the Supporting Information). The structures were relaxed to a local minimum in intermolecular lattice energy, calculated from the FIT54 exp-6 repulsion-dispersion potential and atomic charges, fitted to electrostatic potential around the PBE0/aug-cc-pVTz charge density using the CHELPG scheme.⁵⁵ For each of the two monohydrate searches the 10000 lowest energy structures were refined using DMACRYS⁵⁶ with a more realistic, distributed multipole model⁵⁷ for the electrostatic forces which had been derived using GDMA2⁵⁸ to analyse the PBE0/aug-cc-pVTz charge density.

The orientation of the amino group (planar vs. pyramidal orientation) in the most stable structures (12 kJ mol⁻¹ range with respect to the global minimum structure) of the two compounds was optimized with the program CrystalOptimizer.⁵⁹ Conformational energy penalties and isolated molecule charge densities were computed at the PBE0/aug-cc-pVTz level of theory.

In a final step DFT-D calculations carried out with the CASTEP plane wave code⁶⁰ using the Perdew-Burke-Ernzerhof (PBE) generalized gradient approximation (GGA) exchange-correlation density functional⁶¹ and ultrasoft pseudopotentials,⁶² with the addition of a semi-empirical dispersion correction, either the Tkatchenko and Scheffler (TS)⁶³ or Grimme06 (D2).⁶⁴ For more details see section 2.1. of the Supporting Information.

The same computational method, DFT-D, was used to model mixed cytosine/5-flucytosine monohydrate structures (generated by manually exchanging the C5 hydrogen and fluorine atoms) and isostructural dehydrate structures (manual removal of water molecules). Overall, 28 mixed **fh1-I** and 5 mixed **ch1** structures were generated in addition to the two experimental (**ch1**, **fh1-I**) and two isomorphous structures (cytosine in **fh1-I** and 5-flucytosine in **ch1**). For more details, see section 2.5 of the Supporting Information.

3 Results

3.1 Preparation of Cytosine Solid Forms

The two anhydrate polymorphs, **C-I** and **C-II**, as well as monohydrate of cytosine (**ch1**) were reproduced in the course of this study. The hydrate **ch1** was obtained in cooling crystallization and evaporation experiments from water or organic solvent/water mixtures with a water activity (a_w) > 0.45. Dehydration of **ch1** and cooling crystallization experiments using dry solvents resulted in **C-I**. The second anhydrate polymorph, **C-II**, was obtained concomitantly with **C-I** in sublimation experiments.

3.1.1 Moisture Dependent Stability of Cytosine Solid Forms—The stability of **C-I** and **ch1** was investigated under different moisture conditions in the range of 0 to 95% RH at 25 °C. The gravimetric moisture sorption/desorption isotherms (Figure 2a) show that both solid forms, **C-I** and **ch1**, are stable within a wide range of humidity conditions. Hydration of **C-I** to **ch1** occurs at RH = 70% in a single step. Complete transformation of **C-I** to **ch1** was achieved within a day at 92 % RH. The dehydration of **ch1** to **C-I** takes about three

days and less than a day at 10% and 0% RH, respectively. The distinct steps and hysteresis between the sorption and desorption isotherms are characteristic of a stoichiometric hydrate. 65,66

The slurry method was applied to determine the critical water activity (a_w) of **C-I** $\leftrightarrow$ **cH1**. Anhydrous cytosine was added to methanol/water mixtures of various compositions (Section 1.3 of the ESI) and equilibrated under stirring for ten days. Samples were withdrawn periodically and analyzed with PXRD. In methanol/water mixtures with $a_w = 0.42$, pure **C-I** was exclusively obtained as solid form (Figure 2b). At $a_w = 0.43$ **cH1** was determined as the resulting (stable) form, suggesting that the equilibrium water activity of the cytosine **C-I** $\leftrightarrow$ **cH1** transition is about 0.425 at 25 °C. The a_w study illustrates that in the case of the cytosine **C-I** $\leftrightarrow$ **cH1** system the thermodynamic equilibrium is situated almost in the centre of the hysteresis range observed in the moisture/sorption desorption experiments (Figure 2a).

3.1.2 Temperature Dependent Stability of Cytosine Monohydrate—The dehydration process of **cH1** was monitored with HSM, DSC and TGA and key thermodynamic data are given in Table 1. To investigate the influence of the atmospheric conditions on the dehydration process, different experimental setups were chosen: dry and silicon oil embedded HSM preparations, DSC runs using open (pinholed) or sealed (closed) pans, and different heating rates.

The dehydration of **cH1** starts at temperatures > 50 °C, which is indicated by spots appearing on the surface of the hydrate crystals (Figure 3a). These spots correspond to the nucleation centres of **C-I**. The number of nucleation centres increases with temperature resulting in very small crystals. Based on the hot-stage microscopic investigations it can be concluded that the dehydration reaction below the peritectic dissociation involves a low nucleation but high growth rate. After dehydration the particles are completely opaque due to the formation of numerous small crystallites, but the outer shape of the original hydrate crystal is maintained. The latter, “pseudomorphosis”, is typical for the dehydration mechanism of stoichiometric hydrates. The formation of bubbles is observed in low viscosity silicon oil preparations (1000 mPa s, 25 °C) and heating rates < 5 °C min⁻¹ and confirms the loss of solvent (water) molecules. Hydrate crystals embedded in higher viscosity silicon oil (1,000,000 mPa s, 25 °C) and heated at higher rates (> 5 °C min⁻¹) show a different behavior (Figure 3b). At temperatures > 90 °C **C-I** nucleates on the surface of the hydrate crystal. At the peritectic dissociation/transformation temperature the forming nuclei of the anhydrate crystals grow at a very high rate. Because of the fast nucleation and growth rate of the anhydrate crystals, the incongruent fusion process of the hydrate crystals is hardly observable at the applied heating rates. The peritectic dissociation occurs at ~ 97 °C (Table 1). Above the peritectic temperature the hydrate cannot exist. Upon further heating, the sublimation of the two polymorphs **C-I** and **C-II** can be observed with HSM at temperatures > 240 °C (Figure S41 of the Supporting Information) and the concomitant decomposition and melting process of the two forms occurs from about 300 °C (melting above 320 °C).

The TGA curve (Figure 3c) shows a one-step loss of one mol of water per mol of cytosine. Dehydration under N₂ purge (TGA) was completed at temperatures < 60 °C. In a pinholed DSC pan, the dehydration process is observed in the temperature range from 60 to 75 °C.

The desolvation process below the peritectic temperature, $\text{cH1}_{(s)} \rightarrow \text{C-I}_{(s)} + \text{H}_2\text{O}_{(g)}$, requires $50.7 \pm 0.4 \text{ kJ mol}^{-1}$ ($\Delta_{\text{dehy}}H$, Table 1). In contrast, in a sealed pan (isochoric conditions, composition of the binary system remains unchanged) the peritectic transformation/dissociation of **cH1** to **C-I** is observed at $97 \pm 0.3 \text{ }^\circ\text{C}$, with a heat of dissociation ($\Delta_{\text{diss}}H$) of $9.7 \pm 0.4 \text{ kJ mol}^{-1}$. The melting point of **C-I** could be measured at $324.5 \pm 0.5 \text{ }^\circ\text{C}$, using heating rates of $50 \text{ }^\circ\text{C min}^{-1}$. Decomposition of the compound and melting overlapped. No detailed experimentation was carried out to study the thermal decomposition and its impact on the melting temperature and heat of fusion. Upon cooling the cytosine solid forms from room temperature to $-120 \text{ }^\circ\text{C}$ no phase transformations were observed.

The temperature-composition phase diagram of the system cytosine/water (Figure 4) was constructed using DSC data for mixtures of **cH1** with pure water and **C-I** and shows the typical behavior of an incongruent melting hydrate with a peritectic temperature at $97 \text{ }^\circ\text{C}$ (open circles) and an eutectic (monotectic) between **cH1** and water at $0.2 \pm 0.3 \text{ }^\circ\text{C}$ (black circles). Below the peritectic temperature ($97 \text{ }^\circ\text{C}$, at roughly ambient pressure) the hydrate is thermodynamically stable in a saturated solution or at saturated water vapor pressure.

3.1.3 Enthalpy of **cH1 $\leftrightarrow$ **C-I** Transformation**—The enthalpy of the **cH1** $\leftrightarrow$ **C-I** transition can be estimated from DSC and IC (RH-perfusion experiments). Using open DSC dehydration data and applying Hess's law, the known enthalpy value for the vaporization of water at the dehydration temperature (peak maximum, $T_{\text{dehy}} \approx 75 \text{ }^\circ\text{C}$, $\Delta_{\text{vap}}H_{\text{H}_2\text{O}} = 41.809 \text{ kJ mol}^{-1}$ 67) can be subtracted from the measured heat of dehydration ($\Delta_{\text{dehy}}H$), according to eq. (1), resulting in an estimation of the heat change ($\Delta_{\text{trs}}H$) upon hydrate to anhydrate transformation.

$$\Delta_{\text{trs}}H_{\text{cH1} - \text{CI}} = \Delta_{\text{dehy}}H_{\text{cH1} - \text{CI}} - \Delta_{\text{vap}}H_{\text{H}_2\text{O}} \quad (1)$$

The enthalpy of this reaction was calculated to be $8.8 \pm 0.2 \text{ kJ mol}^{-1}$ (Table 1).

DSC experiments in (hermetically) sealed pans can provide transformation temperatures and transformation enthalpies. In DSC experiments of hydrates prepared in (hermetically) sealed pans, any water that is released from the hydrate remains in the system (isochoric condition). In the rare case of two hydrates with the same stoichiometry (polymorphic hydrate), the transformation enthalpy between these hydrates can be estimated from the measured dissociation enthalpies under isochoric conditions. However, in the case of hydrates with different water stoichiometries, the measured enthalpy value cannot be simply used for the calculation of the transition temperature. This is because the total heat of the dissociation process observed in hermetically sealed DSC containers also includes an unknown heat contribution that originates from the dissolution of a part of the solid in the released water (heat of solution). The energy fraction of this dissolution process is naturally smaller for badly water soluble than for better soluble substances. In the case of the **cH1** to **C-I** transformation a heat of $9.7 \pm 0.4 \text{ kJ mol}^{-1}$ ($\Delta_{\text{diss}}H$, Table 1) was determined, a value slightly higher than the one derived from the pure dehydration peak in open DSC pans. This

difference is not surprising considering that a part of **C-I** dissolves in the released water under isochoric conditions.

RH-perfusion experiments (25 °C) produced an enthalpy of dehydration value ($\Delta_{\text{dehy}}H$) of $51.7 \pm 0.3 \text{ kJ mol}^{-1}$ (Table 1). Using eq (1) and the enthalpy of vaporization of water at 25 °C ($43.99 \text{ kJ mol}^{-1}$), a value of $8.7 \pm 0.3 \text{ kJ mol}^{-1}$ is obtained for the transition of **cH1** to **C-I**. RH perfusion IC allows also the measurement of the heat of hydration ($\Delta_{\text{hy}}H$), which should be equal to the heat of dehydration with the opposite sign since the magnitude of the heat of condensation of water ($\Delta_{\text{cond}}H_{\text{H}_2\text{O}}$) is equal to the heat of vaporization of water. Having said this, the transition energy of **C-I** to **cH1** can be estimated according to eq (2):

$$\Delta_{\text{trs}} H_{\text{CI} - \text{cH1}} = \Delta_{\text{hy}} H_{\text{CI} - \text{cH1}} - \Delta_{\text{cond}} H_{\text{H}_2\text{O}} \quad (2)$$

This resulted in a reaction enthalpy for the hydrate formation ($\Delta_{\text{hy}}H$) of $-8.9 \pm 0.4 \text{ kJ mol}^{-1}$. Thus, the experimentally obtained values for the **C-I** $\leftrightarrow$ **cH1** transition are in excellent agreement with one another (8.7 to 8.9 kJ mol^{-1}) though they were determined with different methods (DSC and IC).

The hydrate $\leftrightarrow$ anhydrate transition enthalpy can also be estimated by comparing the lattice energy, E_{latt} , of the hydrate to the energies of the anhydrate and ice, according to eq. (3). The lattice energy corresponds to the energy required to separate the static lattice into infinitely separated molecules in their lowest-energy conformation.

$$\Delta_{\text{trs}} U_{\text{CI} - \text{cH1}} \approx -E_{\text{latt}}(\text{cH1}) - (-E_{\text{latt}}(\text{CI})) - (-E_{\text{latt}}(\text{ICE})) \quad (3)$$

Using the lattice energies of the experimental hydrate and anhydrate structures (Table 1) and a value of -59 kJ mol^{-1} for ice^{68,69} (the used functional is known to overbind the ice crystal structures^{70,71}) gives an average heat of transformation of $10.8 \pm 0.5 \text{ kJ mol}^{-1}$. The lattice energy calculations slightly overestimate the heat of transformation.

3.2 5-Flucytosine Monohydrate I and Anhydrates

The systematic slurry and dehydration experiments performed in our study successfully reproduced the two anhydrate polymorphs (**F-I** and **F-II**) and the hydrates. The hemipentahydrate (**fH2.5**) and **fH1-I** were obtained in slurry experiments in water at temperatures $< 15 \text{ °C}$ and $> 15 \text{ °C}$, respectively. Evaporation experiments from THF lead to the second 5-flucytosine monohydrate (**fH1-II**), albeit concomitantly with **fH1-I**. Reproduction of the DMSO (DUKWAI36), dimethyl acetamide (DUKWEM36) and methanol (MEBQOA30) solvates were attempted and successful. In addition, two novel solvates, 5-flucytosine hemi-ethanol and mono-dimethylformamide solvates, emerged in the course of our work. The desolvation of the solvates resulted in the known anhydrates, thus, the solvates were not further characterized. Phase pure **F-I** can be prepared in slurry experiments using organic (mixed) solvents at a water activity (a_w) 0.4 and temperatures 10 °C . Dehydration of **fH1-I** at temperatures $< 25 \text{ °C}$, using P_2O_5 or vacuum, results in **F-II**.

For more details, see sections 1.3 and 1.9 of the Electronic Supporting Information. **F-II** is metastable at room temperature but shows a high kinetic stability. Therefore, no transformation to **F-I** was observed in samples stored for four years.

3.2.1 Moisture Dependent Stability of 5-Flucytosine Solid Forms—The sorption isotherms were recorded for the two anhydrate polymorphs and show a single-step hydration process which starts for **F-I** at RH values $\geq 85\%$ (Figure 5a) and for **F-II** at RH $> 60\%$ (Figure 5b). Both sorption experiments resulted in **fh1-I**. The thermodynamically stable anhydrate polymorph shows the higher stability with respect to moisture than the metastable form **F-II**. Dehydration of **fh1-I** occurs at a RH $\geq 35\%$ and **F-II** was predominantly observed as product in the desorption experiments. However, also traces of **F-I** were identified in dehydration products prepared at low RH conditions. To obtain phase pure **F-II** from **fh1-I**, the dehydration must be performed at lowest RH conditions using a strong desiccant such as P_2O_5 or by applying vacuum.

Water activity experiments, using the slurry method (methanol/water mixtures, section 1.3 of the Electronic Supporting Information), revealed that at $a_w = 0.40$ the anhydrate (**F-I**) is the thermodynamically most stable form whereas at $a_w = 0.41$ **fh1-I** was observed in the slurry, suggesting that the equilibrium water activity of the transition 5-flucytosine **F-I** $\leftrightarrow$ **fh1-I**, is around 0.405 at 25 °C. In the case of 5-flucytosine, the kinetic control of the anhydrate to hydrate transformation (hydration) is significantly stronger than in the dehydration process. This is indicated by the fact that the hydration occurs at an atmospheric moisture condition of $a_w > 0.8$ ($> 80\%$ RH) for **F-I** and thus far above the true equilibrium $a_w \sim 0.405$ ($a_w \approx 0.4$). The dehydration of **fh1-I** occurs at an atmospheric moisture condition of $a_w = 0.35$, which is very close to the equilibrium state ($a_w \sim 0.405$) and therefore indicates a significantly weaker kinetic control for this process compared to hydration.

3.2.2 Temperature Dependent Stability of 5-Flucytosine Monohydrate I—The dehydration process of **fh1-I** observed between 65 and < 90 °C, as seen with HSM (Figure 6a), is governed by a nucleation and growth mechanism. Dark spots emerging at the surface and crystal defects of **fh1-I** indicate nucleation centres of **F-I** and **F-II**. The ratio of **F-I** increases with increasing dehydration temperature. Though the number the nucleation rate slightly increases with temperature, the overall dehydration process is dominated by the high growth rate of the limited number of nucleation centres. Similar, to the dehydration of **ch1**, the process results in the formation of homogeneously sized anhydrate crystals, with the original shape of the **fh1-I** crystal maintained (pseudomorphosis), typical for the dehydration of a stoichiometric hydrate. By embedding the hydrate crystals in high viscosity silicon oil it is possible to monitor the incongruent melting of **fh1-I** between 83 and 86 °C. At the peritectic temperature the hydrate crystals fuse partially, and nucleation and growth of **F-I** (Figure 6b) occurs simultaneously. The process is accompanied by the release of water vapor as indicated by the formation of bubbles in an oil embedding. Upon further heating strong sublimation of **F-I** occurs at temperatures $> 230^\circ\text{C}$ and melting with decomposition is observed above 300 °C. Low temperature DSC experiments for **F-I**, **F-II** and **fh1-I** (room temperature to -120 °C) did not indicate phase transformations suggesting that potential low temperature forms do not form in this temperature range by solid-solid transformation.

The TGA curve of **fH1-I** (Figure 6c) shows a simple one-step dehydration process with a mass loss that corresponds to one mol of water per mol of 5-flucytosine. In a pin-holed DSC capsule (Figure 6c, DSC open) the dehydration process is indicated by a broad endothermic peak between 20 and 60 °C with a heat of dehydration ($\Delta_{\text{dehy}}H$) of $53.1 \pm 0.5 \text{ kJ mol}^{-1}$. Upon further heating, the dehydration product (**F-II**), showed an endothermic event around 170 °C, which is caused by the solid-solid phase transition of **F-II** to **F-I**. The measured heat of this transition is $0.55 \pm 0.5 \text{ kJ mol}^{-1}$, indicating that the polymorphic pair **F-I/F-II** is enantiotropically related,⁷² with **F-I** being the thermodynamically stable polymorph at (confirmed by slurry experiments) and above room temperature, while **F-II** is a low temperature stable form. The peritectic dissociation/transformation of **fH1-I** to **F-I** was measured in closed DSC capsules at $84.2 \pm 0.5 \text{ °C}$ with a heat of dissociation/transformation of $7.8 \pm 0.1 \text{ kJ mol}^{-1}$.

3.2.3 Enthalpy of **fH1-I** $\leftrightarrow$ Anhydrate Transformation and **F-II** to **F-I**

Transformation—From the enthalpy of dehydration of **fH1-I** to **F-II** (see Table 1) obtained from heating experiments in open DSC crucibles, and the enthalpy of vaporization of water at the dehydration temperature ($T_{\text{dehy}} \approx 70 \text{ °C}$, $\Delta_{\text{vap}}H_{\text{H}_2\text{O}} = 42.034 \text{ kJ mol}^{-1}$ ⁶⁷) the heat of hydrate to anhydrate transition (**fH1-I** to **F-II**) was calculated according to equ. (1) to be $9.6 \pm 0.2 \text{ kJ mol}^{-1}$. The value of $7.8 \pm 0.2 \text{ kJ mol}^{-1}$ ($\Delta_{\text{diss}}H$, Table 1) obtained for the peritectic decomposition peak in sealed DSC pans, corresponds to the heat of dissociation ($\Delta_{\text{diss}}H$) of **fH1-I** to **F-I**. However, this value also includes the dissolution process of some portion of **F-I** in the free water liberated from the monohydrate, why the $\Delta_{\text{diss}}H$ value does not directly correspond to the transition enthalpy ($\Delta_{\text{trs}}H$) of the hydrate to the metastable polymorph.

The $\Delta_{\text{trs}}H$ value for the **fH1-I** to anhydrate transformation could not be reliably determined using IC (RH-perfusion method), because dehydration at low humidity conditions yields a variable mixture of the two anhydrates **F-I** and **F-II**. However, RH-perfusion IC allowed the determination of the reverse reaction (hydration to **fH1-I**) of both forms from which a transition enthalpy of -10.9 ± 0.3 and $-10.3 \pm 0.2 \text{ kJ mol}^{-1}$, was obtained for the transition of **F-I** and **F-II** to **fH1-I**, respectively. The difference between these values (0.6 kJ mol^{-1}) corresponds to the enthalpy difference between the two polymorphs. A similar value (0.55 ± 0.02) was measured directly in DSC experiments for the solid-solid transition of **F-I** and **F-II** (see also Table 1), which demonstrates the validity of the approach.

The energy differences (ΔU) derived from the lattice energy calculations for the hydrate to anhydrate transformations are in reasonable agreement with the experimental values. Although, based on the lattice energy calculations, using different dispersion corrections (PBE-TS vs. PEB-D2), it was not possible to derive the thermodynamic stability order of the two anhydrate polymorphs at 0 K. The PBE-TS calculations correctly predicted the stability order and the computed energy value of $\Delta_{\text{trs}}U_{\text{F-II to F-I}}$ of 0.5 kJ mol^{-1} is in excellent agreement with the experimental value. The PBE-D2 calculations inverted the stability order, i.e. **F-I** was wrongly calculated to be 2 kJ mol^{-1} more stable than **F-II**.

3.3 Cytosine and 5-Flucytosine Monohydrate Crystal Energy Landscapes

3.3.1 Comparison of Experimental Monohydrate Structures—The single crystal structures of **cH1**,^{28,31–35} **fH1-I**^{30,37,38} and **fH1-II**³⁰ have already been determined and published. The monohydrate **cH1** crystallizes in the monoclinic space group $P2_1/c$ with one cytosine and one water molecule in the asymmetric unit. The cytosine molecules are hydrogen-bonded through $N1-H\cdots N3$ and $N7-H\cdots O$ into parallel ribbons (RM130 [graph sets 73 C1,1(4), C1,1(6), R2,2(8), Figure 7a]. The ribbons propagate along the direction of the b axis. Adjacent ribbons are related by the c glide symmetry and the perpendicular separation of the ribbons is 3.2 Å. The water molecule forms three strong hydrogen bonds, two $Ow-H\cdots O$ and one $N7-H\cdots Ow$, leading to an approximately trigonal planar coordination of the water molecule (Figure 7b)^{28,31–35} and linking the cytosine molecules into a three-dimensional hydrogen-bonding network.

The **fH1-I** polymorph crystallizes in the monoclinic space group $P2_1/c$, with two 5-flucytosine and two water molecules in the asymmetric unit. The 5-flucytosine molecules are, as in **cH1**, hydrogen-bonded through $N1-H\cdots N3$ and $N7-H\cdots O$ into parallel ribbons, forming the RM1 ribbon motif (Figure 7c). The RM1 ribbon motif propagates along the crystallographic b axis. Adjacent ribbons are related by the c glide symmetry and the perpendicular separation of the ribbons is 3.2 Å. The relative orientation of the adjacent and stacked RM1 differ in the **cH1** and **fH1-I**, leading to different two- and three-dimensional packing arrangements. Thus, the two monohydrate structures share only the RM1 ribbon motif and therefore show only one-dimensional structural similarity.³⁹ The first water molecule forms four strong hydrogen bonds, acting twice as hydrogen-bonding donor and twice as hydrogen-bonding acceptor ($Ow-H\cdots Ow'$, $Ow'-H\cdots Ow$, $Ow-H\cdots O$ and $N7'-H\cdots Ow$); the second independent water molecule forms three strong ($Ow-H\cdots Ow'$, $Ow'-H\cdots Ow$, $Ow'-H\cdots O$) and one slightly weaker, i.e. longer, hydrogen bond ($N7-H\cdots Ow'$). Overall, the two water molecules show a comparable tetragonal coordination. The two independent water molecules form cyclic tetramers and the tetramers are arranged in channels parallel to the b -axis. The tetramers link the RM1 ribbon motifs into a 3D hydrogen-bonding network structure. In both, **cH1** and **fH1-I**, the RM1 motifs have no strong hydrogen-bonding interaction with adjacent ribbons.

The second 5-flucytosine monohydrate polymorph, **fH1-II**, crystallizes in the triclinic space group $P-1$, with each one 5-flucytosine and one water molecule in the asymmetric unit. Energy minimizations of the experimental structure, using CASTEP (for details on the modelling see section 2.1.5 of the Supporting Information), confirmed that one of the water protons is disordered over two positions, with adjacent water molecules alternating in its disordered water proton position. **fH1-II** is the only experimental structure of cytosine and 5-flucytosine that does not form the RM1 ribbon motif. Instead, it has the RM2 ribbon motif (Figure 7e). The **fH1-II** structure does not show any structural similarity with **fH1-I** or **cH1**. The water molecule exhibits a tetragonal coordination, hydrogen bonds to two 5-flucytosine molecules ($Ow-H\cdots O$, $N7-H\cdots Ow$) and to two adjacent water molecules ($Ow-H\cdots Ow$ and $Ow\cdots H-O$), and is located in channels parallel to the a -axis.

3.3.2 Computationally Generated Monohydrate Structures—The

computationally generated $Z'=1$ cytosine (Figure 8a&b) and 5-flucytosine monohydrate (Figure 8c&d) crystal energy landscapes have each several thermodynamically feasible crystal structures in the energy range expected for polymorphs.⁷⁴ The known **cH1** structure corresponds to the lowest energy structure on Figure 8a. The energy gap between the global energy minimum structure (**cH1**) and the second most stable computed structure was calculated to be 3.2 kJ mol^{-1} (PBE-D2). Sixteen $Z'=1$ structures were found within eight kJ mol^{-1} of **cH1** (Figure 8a).

The two 5-flucytosine monohydrate polymorphs lie outside the scope of the $Z'=1$ calculations. Therefore, the experimental structures (**fH1-I**: $Z'=2$ and **fH1-II**: P-1 structure transformed to P1 to resolve the proton disorder, resulting in an ordered $Z'=2$ structure) were energy minimized using the same settings as used for the generation of the crystal energy landscapes and added to Figure 8b&d. The experimental hydrate structures were then found as global energy minimum (**fH1-II**) and third lowest energy structure (**fH1-I**) on the combined crystal energy landscape (Figure 8c). The energy difference between the two polymorphs was calculated to be 3.29 kJ mol^{-1} and 4.39 kJ mol^{-1} using PBE-TS and PBE-D2, respectively. 17 structures were found within the 8 kJ mol^{-1} range with respect to **fH1-II**.

To be able to directly compare the computed crystal energy landscapes of the two compounds, the cytosine landscape was complemented with the two experimental 5-flucytosine monohydrate structures: F-atoms were replaced with H-atoms and the structures minimized. Analysis of the packing, hydrogen-bonding motifs and water void-space in the lowest energy structures (10 kJ mol^{-1} range) revealed the delicate balance of hydrogen-bonding between host molecules and between host and guest molecules and furthermore, the influence on $\text{H} \leftrightarrow \text{F}$ exchange on the crystal packing. Several distinct hydrogen-bonding chain/ribbon motifs were identified among the lowest-energy structures (Figure 8e). Two ribbon motifs, RM1 and RM2, four chain motifs (C1-4) involving no water molecules, and seven chain motifs with water molecules bridging the host molecules (CW1-7) are feasible. 47% of the lowest energy cytosine monohydrate structures show RM, 47% CW and only 6% C motifs. The CW motifs show higher packing indices than the other motifs (Figure 8a); the RM structures are spread over a wide PI range, with the experimental structure being densely packed but alternate RM structures showing a lower PI. The three cytosine RM2 structures show the lowest packing indices. The C motif packings, only C1 for cytosine, are all higher energy structures. Overall, the experimental RM1 (38% of the low energy structures) and CW1 (26%) motifs are dominating the cytosine monohydrate crystal energy landscape.

In contrast, the low-energy 5-flucytosine monohydrate structures show a different tendency toward H-bonding motifs. 64% of the structures form the RM motifs, and each 18% the CW or C motifs (Figure 8b&e). The RM2 motif is the most dominant among the lowest energy structures (43% of structures). Similar to Figure 8a, CW structures are densely packed and the RM structures are spread over the entire 5-flucytosine PI range. Again, the C structures are less stable than the RM and CW packings. The presence of F-atoms seems to facilitate the formation/stabilize the RM2 motif.

An analysis of the “water space” (space in the hydrate structures not occupied by the host molecules, Figure 8b&d) of the computed structures revealed that RM1 and RM2 structures show considerable voids/water space. The latter can be related to the fact that in RM structures the water is more likely to be arranged in channels than at isolated sites, as seen in the CW structures, where the water bridges either isolated host molecules or dimers thereof. An exception is **ch1**, where water molecules are located at isolated sites. Overall, a calculated void space of ~14% and higher on Figure 8b&d indicates the presence of a channel hydrate, whereas lower percentage values an isolated site hydrate. Consequently, the cytosine and 5-flucytosine monohydrate energy landscapes differ in the likelihood of formation of hydrate classes, i.e. isolated site hydrates dominate the cytosine (Figure 8b) and channel hydrates the 5-flucytosine landscapes (Figure 8d), in agreement with the experimental isolated site **ch1** and the **fh1-I** and **fh1-II** channel hydrates.

Packing comparisons of the cytosine and 5-flucytosine structures revealed isostructures between the two landscapes. Within the 8 kJ mol⁻¹ range of the lowest energy structures four structures were found on the cytosine and 5-flucytosine crystal energy landscapes. It has to be noted that several of the cytosine and 5-flucytosine monohydrates were found to differ only in proton positions of the water molecules and were counted only once. 5-Flucytosine rank 1 (**fh1-II**), 2 (f1_687, Table S18 of the Supporting Information), 3 (**fh1-I**), and 5 (f3_171) structures can be found as low energy structures on Figure 8a. The 0 K stability order of the isostructural packings on Figure 8a is as follows: **fh1-I** (+3.42 kJ mol⁻¹ with respect to **ch1**) > **fh1-II** (+5.32) > f3_171 (+5.64) > f1_687 (+5.98). Thus, **fh1-I** is the most stable packing seen on both crystal energy landscapes. The isostructural **ch1** packing can also be found on the 5-flucytosine crystal energy landscape (Figure 8c), albeit 15.91 kJ mol⁻¹ higher in energy than **fh1-II**. Driven by these results, that the **fh1-I** is the rank 3 structure on Figure 8c and rank 4 structure on Figure 8a, the calculations suggest that either a cytosine monohydrate polymorph or a cytosine/5-flucytosine monohydrate co-crystal or solid solution exists. Thus, we extended our experimental search space with seeding experiments.

3.4 Solid Solution of the Monohydrates of Cytosine and 5-Flucytosine

3.4.1 Preparation and Characterization—Saturated solutions (at 25 and 85 °C) of cytosine and 5-flucytosine were seeded with **ch1** or **fh1-I** with the aim to produce isomorphic cytosine and 5-flucytosine hydrate structures. Furthermore, mixed saturated solutions of cytosine and 5-flucytosine (molar ratios of 4:1, 2:1, 1:1, 1:2, 1:4, 1:9) were evaporated at 25 °C and slurries of the two compounds (molar ratios of 2:1, 1:1, 1:2 and 1:19) in water were prepared and stirred at 25 °C. Finally, also cooling crystallization experiments from water using different molar ratios (4:1, 2:1, 1:1, 1:2, 1:4 and 1:9) were performed. For more details, see section 1.8 of the Supporting Information.

We were not able to produce a cytosine monohydrate polymorph, which is isostructural with **fh1-I**. However, the PXRD data (Figure 9a) and IR spectra (Figure 9b) of selected seeded evaporation, mixed compound crystallization and slurry experiments bear resemblance with the **fh1-I** data. A closer analysis of the data revealed small, but significant differences between **fh1-I** and the cytosine/5-flucytosine crystallization products. The PXRD pattern of

the 1:1 sample, **ss-c1:f1** hereafter, was successfully indexed to a monoclinic $P2_1/c$ unit cell (25 °C: $a = 7.359(<1)$ Å, $b = 9.400(<1)$ Å, $c = 17.700 (<1)$ Å, $\beta = 101.88(<1)^\circ$, $V = 1198.20(12)$ Å³) with $Z' = 2$. A comparison of the **ss-c1:f1** lattice parameters to the **fh1-I** values (25 °C: $a = 7.506(<1)$ Å, $b = 9.395(<1)$ Å, $c = 17.651 (<1)$ Å, $\beta = 99.15(<1)^\circ$, $V = 1228.86(10)$ Å³) confirms the presence of a new phase. The IR spectra suggest that **ss-c1:f1** and **fh1-I** are isostructural. Differences between the latter two solids can be seen in the vibrational range of CH deformation and CF stretching vibrations (highlighted in yellow in Figure 9b). Independent of the used molar ratio of cytosine and 5-flucytosine (2:1, 1:1, 1:2 and 1:19) only one phase, with a maximum deviation in cell volume of 5.5 Å³, but different intensities in IR spectra, was obtained. It is therefore reasonable to assume that not a molecular compound (what is now commonly denoted as “cocrystal”) but rather that **fh1-I** forms a solid solution with cytosine. Water content determinations of the solid solutions allowed us to deduce the molar ratios of the two compounds.

We have succeeded in solving the single crystal structure of the cytosine/5-flucytosine monohydrate solid solution (1:1 mixture). The data confirm that the mixed crystal is isostructural with **fh1-I** ($P2_1/c$), with each of the two compounds plus two water molecules in the asymmetric unit. The host molecules form the RM1 and the water molecules link the ribbons into a three-dimensional hydrogen-bonded structure. The two water molecules are tetragonally coordinated and arranged in channels along the crystallographic a axis (Figure 10). F $\leftrightarrow$ H exchange occurs for each of the two independent host molecules, with F-position occupancies of 47% and 50%. In **fh1-I** the distance between adjacent F-atoms was found to be 2.643 Å (BIRMEU02). This contact is shorter in **ss-c1:f1**, 2.178 Å, suggesting that every alternate F-atom is replaced by hydrogen, leading to the smaller cell volume in **ss-c1:f1** compared to **fh1-I**.

3.4.2 Moisture Dependent Stability of Cytosine/5-Flucytosine Solid Solutions

—Solid solutions with variable composition were investigated under different moisture conditions in the range of 0 to 95% RH at 25 °C (Figure 11a-d) and contrasted to the sorption/desorption curves of a physical mixture of cytosine and 5-flucytosine (Figure 11e). Desorption of the monohydrate solid solutions occurs at RH values < 25%. Thus, the presence of cytosine in the 5-flucytosine monohydrate stabilizes the structure. The desorption of **fh1-I** already started at higher RH values (< 40 % RH, Figure 5a). Desorption of the monohydrate solid solution resulted in an anhydrous solid solution, independent of the molar ratios used. The anhydrous solid solution seems, based on PXRD and IR measurements, to be isostructural with **F-II** (see sections 1.4 and 1.10 of the Electronic Supporting Information). The anhydrous solid solution (**ssF-II**) is less resistant to moisture than its corresponding **F-II** phase. The solid solution transforms to the monohydrate solid solution at RH > 30%, whereas **F-II** transforms at higher RH values (> 60%). The critical water activity for the solid solution must be between 0.25 and 0.3, which is lower than the a_w value determined for the system **F-I** $\leftrightarrow$ **fh1-I** (0.4). Based on the course of the sorption/desorption isotherms it is possible to differentiate between the presence of a solid solution and a physical mixture of the two compounds. The mixture shows two steps in the dehydration isotherm, one for 5-flucytosine and one for cytosine (Figure 11e).

3.4.3 Temperature Dependent Stability of the Cytosine/5-Flucytosine Solid

Solution—With hot stage microscopy (dry preparation) the dehydration process of **ss-c1:f1** can be noticed at temperatures $> 60\text{ }^{\circ}\text{C}$ and is indicated by a loss of optical transmittance of the hydrate crystals (Figure 12a). The process starts preferentially at the short ends of the crystals and proceeds along the longitudinal axis from both ends. On heating the crystals in low viscosity silicon oil preparations the decrease in transparency occurs gradually and the increasing formation of bubbles is observed from about $90\text{ }^{\circ}\text{C}$ confirming the loss of water (Figure 12b). The dehydrated crystals appear opaque but maintain the original outline (pseudomorphosis).

The TGA curve (Figure 12c) shows that the monohydrate releases water already at $20\text{ }^{\circ}\text{C}$ under the dry N_2 purge. On heating the dehydration process proceeds slowly and even at low heating rates ($2\text{ }^{\circ}\text{C min}^{-1}$) it was not possible to complete the dehydration below the peritectic temperature of the hydrate. In a pin-holed DSC capsule (Figure 12c, DSC open) the dehydration is indicated by a broad endothermic peak, ranging from 25 to $125\text{ }^{\circ}\text{C}$, with a peak maximum at $65\text{ }^{\circ}\text{C}$. Thus, the solid solution releases the water much slower than the isomorphous hydrate **fh1-I**. Dehydration in open DSC crucibles resulted mainly in **ssF-II** with smaller amounts of **ssF-I**, which shows similar PXRD and IR characteristics as **F-I**. Upon further heating an endothermic transformation of **ssF-II** to **ssF-I** is observed, similar to the behavior of **F-II** (see Figure 6c). The characteristics of the anhydrous solid solutions will be addressed elsewhere.

The peritectic dissociation of the hydrate to **ssF-I** is observed by heating the hydrate crystals in a high viscosity silicon oil embedding (HSM) or sealed DSC pans with higher heating rates ($> 10\text{ }^{\circ}\text{C min}^{-1}$, Figure 12c). Depending on the composition of the solid solution the peritectic temperature varies between about 150 and $100\text{ }^{\circ}\text{C}$ (Figure 12d). The hydrate solid solution shows a higher thermal stability (peritectic dissociation) than the **fh1-I** phase.

3.4.4 Modelling Monohydrate Solid Solutions—H $\leftrightarrow$ F exchange was

systematically applied to the experimental monohydrate structures **ch1** and **fh1-I** to produce isostructural cytosine, 5-flucytosine and mixed crystal structures thereof (section 2.5 of the Supporting Information). The structures were minimized as described in section 2.6 (PBE-TS and PBE-D2). The lattice energies are plotted against number of C5 hydrogen atoms in Figure 13a&b. The straight dotted line drawn between the **fh1-I** and **ch1** structures indicates the relative stability of putative mixed (co-crystal, solid solution) structures with respect to the pure components. Structures above the line are estimated to be less stable and therefore unlikely to form in crystallization experiments, structures on or below the line can be expected to form from mixed cytosine and 5-flucytosine crystallizations. None of the mixed **ch1** structures was calculated to be more stable than the **fh1-I** packing. Mixed **fh1-I** structures down to 25% 5-flucytosine were calculated, based on lattice energies, to be more stable than **fh1-I** and **ch1**. Mixed crystals with 5-flucytosine contents of 12.5% and less were computed to be less stable than **ch1**. Thus, based on the calculations on periodic structures mixed **fh1-I** crystal formation should occur if 25% or more 5-flucytosine is present. The fact that different cytosine/5-flucytosine ratios and even more than one structure with the same molar ratio of the two compounds are close in energy and more stable than the

pure components rationalizes the formation of a solid solution rather than a cocrystal. The experimental findings (Figure 13c) are in good agreement with the lattice energy calculations indicating the following: (i) it is neither possible to produce a **ch1** co-crystal nor a solid solution isostructural with **ch1**; (ii) isostructural solid solutions of **fh1-I** only form if more than 20% of 5-flucytosine is present in the crystallization experiments. At lower ratios a mixture of **ch1** and the monohydrate solid solution is obtained.

4 Discussion

The two chemically related compounds cytosine and 5-flucytosine differ only in the substitution of the C5 position. Both compounds are polymorphic (two neat forms each) and form solvates (hydrates). Co-crystallization experiments of the two substances result in the formation of solid solutions (neat and hydrated). With the aim of exploring the influence of the substitution on the crystal packing and the kinetic and thermodynamic stability relationships between practically relevant forms, we have established the role of H- and F-atoms in stabilizing the crystal structures.

4.1 Channel vs. Isolated Site Hydrates: Implications for Phase Stability

According to the structural hydrate calcification system introduced by Morris and Rodriguez-Hornedo,^{3,20} **ch1** is an isolated site hydrate and **fh1-I** a channel hydrate. The relative stability of the hydrates can be derived from the crystal structure and the water sorption/desorption data, which indicate the binding strength of water and the structural accessibility of water molecules from the environment. Commonly, channels hydrates show a more or less continuous and largely nonstoichiometric hydration/dehydration behavior, whereas isolated site hydrates sorb/desorb water in a stepwise manner.⁶⁵ The Hydrate Analyzer tool in Mercury indicates that in the optimized structures water occupies only 2.9% of the void space in **ch1** (isolated sites), whereas in **fh1-I** 17% of space is occupied (channels), leading to a packing index of 76.1 for **ch1** and only 72.7 for **fh1-I** (Figure 8).

In the case of **ch1** the stoichiometric (stepwise) sorption/desorption behavior (Figure 2a) can be rationalized from the structural features, as the removal of the water molecules requires a disruption of the hydrate crystal structure. From the channel-like location of the water molecules in the structure a nonstoichiometric behavior might be expected for **fh1-I**. The latter would imply, that the loss of the water molecules from the channels causes only weak changes of the hydrate structure and more important that the water content may adopt variable values depending on the external conditions (moisture and temperature), as seen for example for brucine HyA14. However, from the gravimetric moisture sorption/desorption experiments it is evident that, despite being a channel hydrate, **fh1-I** shows a similar hydration/dehydration behavior (Figure 5a) as observed for **ch1**. The dehydration leads to a distinct crystal packing (**C-I** and/or **C-II**, depending on the dehydration conditions, see section 1.9 of the Supporting Information) instead of an isomorphic dehydrate. The reason for this stoichiometric dehydration behavior may be explained by the role of water as structure stabilizer and the number and strengths of hydrogen bonds to the water molecules. All donor and acceptor groups of cytosine and 5-flucytosine in the hydrates **ch1** and **fh1-I** form strong hydrogen bonds. However, the water molecules in the two hydrate structures

exhibit different hydrogen bonding patterns. In **cH1** water forms only three strong hydrogen bonds, which is the most common hydrogen-bonding pattern around water molecules in hydrates (DDA: D - donor bond, A – acceptor bond, ~50% of all CSD hydrate structures⁷⁵). In contrast, the **fH1-I** water molecules form four hydrogen bonds, the DDAA interaction motif (~17% of the CSD hydrate structures show this motif, the second most popular⁷⁵).

Lattice energy calculations of the **cH1** and **fH1-I** monohydrate and the isostructural dehydrate structures (removal of water molecules and optimizing the cytosine or 5-flucytosine network of the empty hydrate structures) revealed that in **cH1** the water molecules contribute less than 10% to the lattice energy of the hydrate. In contrast, over 40% of the lattice energy can be attributed to the water interactions in **fH1-I** (Table 2), which highlights the importance of the strong water···water interactions for maintaining the structural network and explains why the loss of water results in a rearrangement of the RM1 motif to a more stable and denser packing.

Water activity measurements (Figure 2b and Figure 5c) revealed that the two hydrates, **cH1** and **fH1-I**, show similar stability ranges. The thermodynamic equilibrium of the cytosine hydrate $\leftrightarrow$ anhydrate transition lies around 42.5% RH, whereas that of 5-flucytosine is around 40.5% RH. On the other hand, the gravimetric moisture sorption/desorption studies (Figure 2a and Figure 5a&b) reflect the kinetic control of the process, *i.e.* indicate the structural resistance for the water molecules to leave the hydrate structure (dehydration) or to form the hydrate. The desorption process of the channel hydrate **fH1-I** occurs at 35% RH which is close to the thermodynamic equilibrium condition (40% RH) and indicates that the kinetic barrier of the release of water molecules from the channel hydrate **fH1-I** is low. The isolated site hydrate **cH1**, dehydrates at atmospheric conditions of 10% RH (kinetic value), which is far below the thermodynamic equilibrium (42.5% RH). This larger difference suggests that the release of water molecules from the isolated sites in the **cH1** crystal lattice requires a higher energy contribution (activation) than from the channel hydrate **fH1-I**.

The experimentally determined energies for the heat of hydrate to anhydrate transformations (DSC and IC) resulted in values of similar magnitude for the two hydrates, 10 – 11 kJ mol⁻¹ for 5-flucytosine (depending on the dehydration product) and ~9 mol⁻¹ for cytosine. This suggests that the two hydrates are of comparable stability. In 5-flucytosine the higher number of water hydrogen bonds compensates for the fact that the structure has a lower packing index and hosts the water in easier accessible channels compared to the isolated site hydrate of cytosine, which lacks one strong hydrogen.

4.2 Molecular-Level Understanding of the Influence on F- and H-Atoms on Cytosine and 5-Flucytosine (Monohydrate) Crystal Packing

The comparisons of the experimental cytosine and 5-flucytosine solid form landscapes and their computationally generated monohydrate crystal energy landscapes allow to unravel the influence of the C5 substitution on their solid state forms and properties thereof. All experimental cytosine structures show the RM1 ribbon motif except one 5-flucytosine structure (**fH1-II**), which shows the motif RM2 (see section 1.1 of the Supporting

Information). Thus, neither water or solvent molecules (DMSO, dimethyl acetamide, methanol and 2,2,2-trifluoroethanol) can break the strong ribbon motifs formed by the host molecules only. In each of the two motifs (RM1 and RM2) one hydrogen-bonding donor group (amino hydrogen) and one hydrogen-bonding acceptor (urea oxygen) is exposed, enabling the interaction either between ribbon motifs themselves, as seen in the anhydrates, or with guest molecules such as solvents. The latter not only explains the reason for solvate/hydrate formation but also why, besides the stable monohydrates, also stable anhydrates form.

The computed crystal energy landscapes give us insight into all feasible structures at the molecular level,^{76–78} unravelling which hydrogen-bonding motifs and molecular arrangements are preferred or possible⁷⁹ for a substance. In the case of hydrates also the role of water can be rationalized from the crystal energy landscape (isolated⁸⁰ vs. channel, 14 space filling⁸¹ vs. integrated in the hydrogen bonding network,⁸⁰ etc.). Figure 8a-d clearly indicates the influence of the C5 substitution on the hydrogen-bonding and the packing in potential monohydrate structures. For cytosine RM1 and CW1 (see Figure 8e) are the dominating motifs in the lowest energy structures on the crystal energy landscape, whereas in 5-flucytosine structures RM2 is the preferred motif. Furthermore, cytosine monohydrates are generally denser packed with water molecules being more likely located at isolated sites whereas 5-flucytosine hydrates form low energy packing's, where water is mostly located in channels. We would not have expected such differences in the structural accommodation of water molecules (channel and isolated site) from the molecular features alone.

4.3 Value of Computationally Generated Crystal Energy Landscapes in Solid Form Screening and Characterization

The biggest problem in solid form screening is that it is impossible to cover all crystallization conditions^{82–87} that may lead to (novel) solid forms and there is never a 100% guarantee that all relevant solid forms or even the most appropriate form with respect to stability, manufacturing and biopharmaceutics have/has been found in an experimental screen. Given the recent successes in CSP^{88,89} it is fair to say that the computational search for solid forms can considerably support experimentalists in designing a successful solid form screen and to select targeted crystallizations strategies. In this study, the calculation of the cytosine and 5-flucytosine monohydrate structures, despite searching only for $Z'=1$ hydrates, suggested additional crystallization experiments which resulted in the discovery of previously unknown solid solution(s). Therefore, this study adds to the steadily growing list of successful implementations of CSP in (industrial) screening or characterization programmes (e.g., 42,76,78,90–92). It has to be emphasized that the computational screen alone cannot replace the numerous experiments as it is yet impossible to cover the entire range of possible solid state forms including hydrates and solvates with different host to solvent ratios or other binary crystals and salts. The calculations don't tell us directly how to produce feasible structures, but may help to perform more efficient crystallization strategies. The strength of the calculations is that they can indicate and rationalize (complex) crystallization behavior (polymorphism,^{79,93} intergrowth,⁶⁶ etc).

For cytosine and 5-flucytosine one may have expected a similar crystallization and solid state behavior. However, from the structures that have been reported in the literature so far one would not have foreseen an isostructural behavior between the two compounds. The computed cytosine monohydrate crystal energy landscape suggests that isostructurality between cytosine and 5-flucytosine hydrates is possible and suggested that seeding experiments should lead to either a monohydrate polymorph of cytosine or a co-crystal or a solid solution of the two substances. The fact that lattice energy minimizations with different cytosine/5-flucytosine ratios resulted in stable isostructural structures (Figure 13a&b) indicated the potential existence of a solid solution. Overall, the two computed landscapes suggest that more than one monohydrate structure is thermodynamically plausible for each of the two compounds.

Lattice energy calculations may be used to derive the stability order of polymorphs, albeit at 0 K. For an enantiotropically related pair of forms this implies that the 0 K stability order may not correspond to the RT stability order (e.g. brucine anhydrates⁹⁴). The PBE functional used in this study is known to overbind ice-like interactions.^{84,85} Therefore, the accuracy of the monohydrate lattice energies can be expected to be lower than for anhydrates why we did not speculate whether **fh1-I** and **fh1-II** are monotropically or enantiotropically related (see also section 2.5 of the Supporting Information). The amount of **fh1-II** produced in our studies did not allow us to perform reliable experimental studies (e.g. with solution calorimetry).

The computed monohydrate crystal energy landscapes (Figure 8) indicate low energy structures with alternative hydrogen-bonding motifs (CW1 based hydrates). We have not yet explored special crystallization experiments (e.g. templating) that may allow us to produce such structures and to verify kinetic stability. To enable the formation of these forms a disruption of the RM1 and/or RM2 motifs is required, however, the motifs are likely to be already present in solution. Thus, the experimental challenge remains to achieve sufficient control over molecular self-assembly to be able to promote growth units that can lead to a specific, thermodynamically feasible structure.

5 Conclusions

Despite of their structural relationship the two pharmaceutically important compounds cytosine and 5-flucytosine differ in their tendency towards hydrate formation. Cytosine forms an isolated channel monohydrate (**ch1**), whereas 5-flucytosine exists in four different hydrate structures, one hemihydrate, two monohydrates and one hemipentahydrate. The thermodynamically most stable 5-flucytosine hydrate (**fh1-I**) shows a comparable stability as the cytosine hydrate **ch1** even though it is a less dense structure and hosts the water molecules in open channels. This highlights once more that packing efficiency alone is not a reliable indicator for the stability of a solvated (hydrated) phase, but rather that the interplay of packing efficiency plus strength and number of intermolecular interactions determines the phase stability of a hydrate. The chemical exchange of the C5 cytosine proton with a F-atom not only influences the predominance of hydrogen-bonding motifs in (monohydrate) structures but more important the way in which hydrogen-bonded ribbons are linked (through water) into three-dimensional packings.

The study also highlights that the presence of water molecules in structural channels does not implicate a non-stoichiometric behaviour of a hydrate. Whether the hydrate structure is maintained after a part or all water molecules are withdrawn depends on the stability of the remaining host framework, i.e. the stabilizing role of water in the hydrate. In case of 5-flucytosine, the removal of the water molecules from the channels results in an unstable framework, why a structural reconstruction occurs instead of the formation of an isomorphous dehydrate. The interrelation between the location of water, lattice energy, thermodynamic and kinetic behavior of the hydrate could be well described with the acquired experimental and theoretical data.

Guided by the computationally generated monohydrate cytosine and 5-flucytosine crystal energy landscapes a monohydrate solid solution of the two compounds, with interesting practical features, emerged in this study. The solid solution is isostructural with **fH1-I** and exists only if more than 20% of 5-flucytosine is present. The solid solution shows better thermal- and moisture-dependent stability properties than its pure **fH1-I** structure. Thus, as shown here, computed crystal energy landscapes are an important tool not only for interpreting the range of experimental solid forms but also for proposing thermodynamically feasible forms that have yet to be produced. The next required steps are directed to improvements of existing theoretical approaches in order to increase the reliability for the prediction solid form landscapes, but there is also lots of space to develop experimental strategies that allow us to generate and efficiently characterize specific solid forms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Synopsis

The computationally generated cytosine and 5-flucytosine monohydrate crystal energy landscapes enabled the finding of the first solid solution of the two substances. The solid solution shows better thermal- and moisture-depending stability properties than the structurally related 5-flucytosine monohydrate. The complementarity of the experimental and computational approaches allow to unravel the nature and reason for hydrate formation of the two pharmaceuticals.

Figure 1.
Molecular diagrams of (a) cytosine monohydrate and (b) 5-flucytosine monohydrate.

Figure 2.

(a) Gravimetric moisture sorption and desorption curves of cytosine anhydride (**C-I**) and monohydrate (**cH1**) at 25 °C. The circles represent data points that fulfil the set equilibria conditions (mass change < 0.001 % over 60 min), whereas crosses and dashed lines mark data points where the sample did not reach the equilibrium moisture content within the allowed time limit of 48 h. (b) Phase diagram of cytosine at different water activities in methanol/water mixtures at 25 °C. **C-I** was used as the starting phase.

Figure 3.

(a,b) Photomicrographs of the dehydration process of cytosine monohydrate: (a) Recorded below the peritectic temperature (dry preparation), showing a high growth rate of the anhydrous phase. (b) Preparation in a silicon oil with high viscosity showing an inhomogeneous melting process (melting and fast recrystallization of **cH1** to **C-I**). (c) DSC and TGA thermograms of cytosine monohydrate: open – pinholed pan, closed – sealed pan. TGA curve was recorded at a heating rate of $5\text{ }^{\circ}\text{C min}^{-1}$. $\Delta_{\text{dehy}}H$ – heat of dehydration, $\Delta_{\text{tr}}H$ – heat of hydrate to anhydrate transformation, $\Delta_{\text{diss}}H$ – heat of peritectic dissociation to the anhydrate.

Figure 4.

Temperature-composition phase diagram of the binary system cytosine-water constructed from DSC data. Grey and black points: data points for hydrate-water mixtures; white circles and black diamonds: data points for hydrate-anhydrate mixtures. Diamonds mark the liquidus line and open circles the peritectic dissociation temperatures of the hydrate.

Figure 5.

(a,b) Gravimetric moisture sorption and desorption curves of 5-flucytosine anhydrides (**F-I** and **F-II**) and monohydrate I (**fH1-I**) at 25 °C. The circles represent data points that fulfil the set equilibria conditions (mass change < 0.001 % over 60 min), whereas crosses and dashed lines mark data points where the sample did not reach the equilibrium moisture content within the allowed time limit of 48 h. (c) Phase diagram of 5-flucytosine at different water activities in methanol/water mixtures at 25 °C. A mixture of **F-I** and **F-II** was used as starting material.

Figure 6.

(a,b) Photomicrographs of the dehydration process of 5-flucytosine **FH1-I**: (a) Recorded below the peritectic temperature (dry preparation), showing a high nucleation rate. (b) Preparation in a silicon oil with high viscosity showing an inhomogeneous melting process (melting and fast recrystallization of the hydrate to **F-I**). (c) DSC and TGA thermograms of the monohydrate: open – pinholed pan, closed – sealed pan. TGA curve was recorded at a heating rate of $5 \text{ }^{\circ}\text{C min}^{-1}$. $\Delta_{\text{dehy}}H$ – heat of dehydration, $\Delta_{\text{ts}}H$ – heat of hydrate to anhydrate transformation, $\Delta_{\text{diss}}H$ – heat of peritectic dissociation of the hydrate to the anhydrate.

Figure 7.

(a) Ribbon motif 1 (RM1) of **cH1** cytosine molecules. (b) Packing diagram of **cH1** viewed along the crystallographic *b* axis. (c) Ribbon motif 1 (RM1) of **fH1-I** 5-flucytosine molecules. (d) Packing diagram of **fH1-I** viewed along the crystallographic *b* axis. (e) Ribbon motif 2 (RM2) of **fH1-II** 5-flucytosine molecules. (f) Packing diagram of **fH1-II** viewed along the body diagonal of the unit cell.

Figure 8.

Computed crystal energy landscapes for (a,b) cytosine and (c,d) 5-flucytosine monohydrates. Each symbol denotes a crystal structure. Experimental structures are encircled and isostructural cytosine and 5-flucytosine hydrate structures (diamond symbol) are labelled with c and f. Hy1 – cytosine monohydrate, Hy1-I and Hy1-II – 5-flucytosine monohydrate I and II. In (a,c) the packing index (PI) and in (b,d) the void space, excluding water molecules and calculated using a 1.0 Å probe radius (Mercury), are plotted against lattice energies (E_{latt}). In (e) the hydrogen-bonding motifs found among the low-energy structures (10 kJ

mol⁻¹ range with respect to the lowest energy structures) are shown. RM: ribbon motif, C: chain, CW: water bridged chain.

Figure 9.

(a) Powder X-ray diffractograms and (b) infrared spectra of cytosine monohydrate (**cH1**), 5-flucytosine monohydrate I (**fH1-I**) and a 1:1 solid solution of cytosine and 5-flucytosine monohydrate.

Figure 10.

Void space analysis of cytosine/5-flucytosine (1:1) monohydrate solid solution, excluding the water molecules and showing the water channels along the crystallographic *a* axis. Water space was calculated using the Hydrate Analyzer tool in Mercury and a probe radius and approximately a grid spacing of 1.0 and 0.15 Å, respectively.

Figure 11.

Gravimetric moisture sorption and desorption curves of (a-d) cytosine/5-flucytosine solid solutions and (f) a physical mixture of cytosine and 5-flucytosine at 25 °C. The circles represent data points that fulfil the present equilibria conditions (mass change < 0.001 % over 60 min), whereas crosses and dashed lines mark data points where the sample did not reach the equilibrium moisture content within the allowed time limit of 48 h. The red solid line displays the course of the isotherm if all data points would be in equilibrium while the

dotted line shows the experimental isotherm including non-equilibrium data points. Molar ratios (cytosine:5-fluorocytosine) are given in (a-d).

Figure 12.

(a,b) Photomicrographs of the dehydration process of a cytosine/5-flucytosine monohydrate solid solution: (a) Recorded below the peritectic temperature (dry preparation). (b) Preparation in a silicon oil with low viscosity. (c) DSC and TGA thermograms: open – pinholed pan, closed – sealed pan. TGA curve was recorded at a heating rate of $2\text{ }^{\circ}\text{C min}^{-1}$ from 20 to 140 °C and then kept at 140 °C (isothermal) until weight constancy was achieved. (d) Binary composition diagram: ratio 5-flucytosine vs. peritectic dissociation temperature of the cytosine/5-fluorocytosine monohydrate solid solution (ss).

Figure 13.

(a,b,) Lattice energy comparisons of cytosine **cH1**, 5-flucytosine **fH1-I** and mixed crystals thereof (H ↔ F systematically exchanged). Each symbol denotes a crystal structure. Note, that 0 corresponds to 5-flucytosine, 8 to cytosine, 4 to a 50:50 mixture of cytosine and 5-flucytosine, etc. The **cH1** structure is Z=4 (four host molecules in the unit cell), therefore the factor 2 was used to be able to compare the number of H/F atoms to **fH1-I** (8 molecules in the unit cell). (c) Experimental existence range for cytosine/5-flucytosine monohydrate

solid solutions (blue). “x” denotes the molar ratios for which crystallization experiments were performed.

Table 1
Thermodynamic Data for Cytosine and 5-Fluorocytosine Anhydrate and Monohydrate Solid Forms

Solid Form	C-I	cH1	F-I	F-II	fH1-I
Melting Point (T_{fus}), °C					
Hot-stage microscopy	320 – 325 ^a		> 300 ^a		
Differential scanning calorimetry (DSC)	324.5 ± 0.5 ^b		302 ± 1		
Enthalpy of transformation ($_{\text{trs}}H$)					
kJ mol ⁻¹ at ~170 °C (DSC)				0.55 ± 0.02 (F-II → F-I)	
Lattice energy calculations, -273 °C				-1.2 ± 1.4 (F-II → F-I)	
Peritectic dissociation (T_{diss}), °C					
Hot-stage microscopy		96 – 97			82 – 84
Differential scanning calorimetry (DSC)		97 ± 0.3			84.2 ± 0.2
Enthalpy of dehydration ($_{\text{dehy}}H$)					
kJ mol ⁻¹ at ~75 / 70 °C (DSC, open pan)		50.7 ± 0.4			52.1 ± 0.2
kJ mol ⁻¹ at 25 °C (RH-Perfusion)		51.7 ± 0.3			
Enthalpy of hydration ($_{\text{hy}}H$)					
kJ mol ⁻¹ at 25 °C (RH-Perfusion)	-51.9 ± 0.3		-53.9 ± 0.2	-54.9 ± 0.2	
Lattice energy (E_{latt}), kJ mol⁻¹					
PBE-TS	-180.41	-249.68	-148.80	-149.28	-221.63
PBE-D2 (sp)	-169.84	-239.93	-144.10	-141.99	-213.61
PBE-D2	-170.54	-240.54	-145.75	-143.92	-215.12
Enthalpy of transformation ($_{\text{trs}}H$), kJ mol⁻¹					
kJ mol ⁻¹ at ~90 °C (DSC, closed pan)		9.7 ± 0.4 (cH1 → C-I)			7.8 ± 0.2 (fH1-I → F-I)
kJ mol ⁻¹ at ~75 °C (DSC, open pan)		8.8 ± 0.2 (cH1 → C-I)			9.6 ± 0.2 (fH1-I → F-II)
kJ mol ⁻¹ at 25 °C (RH-Perfusion)	-8.9 ± 0.4 (C-I → cH1)	8.7 ± 0.3 (cH1 → C-I)	-10.9 ± 0.3 (F-I → fH1-I)	-10.3 ± 0.2 (F-II → fH1-I)	
Lattice energy calculations, -273 °C	-10.8 ± 0.5 (C-I → cH1)	10.8 ± 0.5 (cH1 → C-I)	-11.6 ± 2.0 (F-I → fH1-I)	-12.7 ± 0.6 (F-II → fH1-I)	

^a Melting and decomposition occur in the same temperature range.

^b A higher heating rate of 50 °C min⁻¹ was applied.

Table 2
Lattice Energy calculations for cH1 and fH1-I and isostructural dehydrate structures.

Method ^a	Cytosine (E_{latt} / kJ mol ⁻¹)			5-Flucytosine (E_{latt} / kJ mol ⁻¹)		
	cH1	cH1-dehy(sp) ^b	cH1-dehy ^c	fH1-I	fH1-I-dehy(sp) ^b	fH1-I-dehy ^c
PBE-TS	-179.77	-148.28 (82%)	-164.18 (91%)	-221.63	-127.30 (57%)	-149.09 (67%)
PBE-D2	-170.54	-137.19 (80%)	-156.81 (92%)	-215.12	-121.11 (56%)	-143.87 (67%)

^aCASTEP relaxations with dispersion correction (k-points: 0.07 Å⁻¹, cut-off: 780 eV)

^blattice parameters fixed to the lattice parameters of the hydrate structure and atomic positions optimized

^clattice parameters and atomic positions optimized). Percent values are calculated relative to the hydrate lattice energy.