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4-Aminoquinaldine monohydrate polymorphism: Prediction and impurity aided discovery of a difficult to access stable form

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Abstract

Crystal structure prediction studies indicated the existence of an unknown high density monohydrate structure (**Hy1_B**[°]) as global energy minimum for 4-aminoquinaldine (**4-AQ**). We thus performed an interdisciplinary experimental and computational study elucidating the crystal structures, solid form inter-relationships, kinetic and thermodynamic stabilities of the stable anhydrate (**AH I**[°]), the kinetic monohydrate (**Hy1_A**) and this novel monohydrate polymorph (**Hy1_B**[°]) of **4-AQ**. The crystal structure of **Hy1_B**[°] was determined by combining laboratory powder X-ray diffraction data and ab initio calculations. Dehydration studies with differential scanning calorimetry and solubility measurements confirmed the result of the lattice energy calculations, which identified **Hy1_B**[°] as the thermodynamically most stable hydrate form. At 25 °C the equilibrium of the **4-AQ** hydrate/anhydrate system was observed at an *a_w* (water activity) of 0.14. The finding of **Hy1_B**[°] was complicated by the fact that the metastable but kinetically stable **Hy1_A** shows a higher nucleation and growth rate. The presence of an impurity in an available **4-AQ** sample facilitated the nucleation of **Hy1_B**[°], whose crystallisation is favored under hydrothermal conditions. The value of combining experimental with theoretical studies in hydrate screening and characterisation, as well as the reasons for hydrate formation in **4-AQ**, are discussed.

1 Introduction

The objective of solid form screenings of biologically active substances (drugs, agrochemicals), that are manufactured to solid dosage forms, is to find as many solid forms as possible (polymorphs, hydrates, solvates, amorphous phase) and to identify the most suitable form(s) for further development.^{1,2} However, no matter how extensive a screening programme may be, utilising either high throughput methodologies^{3–6} or other widely used (manual) screening strategies,^{7–10} there is no guarantee that all possible forms are found. The most critical and key parameters that influence the occurrence of solid forms are temperature, pressure, moisture, the nature of the solvent of crystallisation (including water

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activity) and supersaturation. Generally, a polymorph screen is performed by crystallising the compound from solvents/solvent mixtures¹¹ and by applying as many different conditions as possible, e.g. varying the rate of cooling, crystallisation temperature, (reverse) anti-solvent addition, solvent evaporation or liquid assisted grinding experiments. Furthermore, crystallisation from the melt,^{12,13} sublimation,¹⁴ moisture sorption/desorption experiments, systematic desolvation (dehydration) studies^{15,16} and pressure crystallisation^{17–19} are successful ways to new crystal forms. Another tool for expanding polymorphism screens is the use of seeds or additives to induce the formation of new crystalline forms by heterogeneous nucleation (e.g. templating, isomorphic seeding).^{20–22}

Tailor made additives of structurally related impurities can significantly modify the nucleation/growth process of crystal forms.^{23–25} An additive can inhibit nucleation by being incorporated into pronuclear clusters and aggregates or by binding to nuclei/crystals. While one polymorph may be affected by the additives, another polymorph may remain unaffected.²⁶ Similarly, the addition of an additive can inhibit the crystal growth by attaching to certain face(s) of crystals. Such an inhibition may block/reduce the formation of one polymorph but favour another form, whose growth rate is less or not affected by the additive.^{27,28,29} Examples for successfully stabilising metastable forms due to (by-product) impurities have been demonstrated for substances such as sulfathiazole,³⁰ L-glutamic acid,²⁵ aspirin,³¹ and progesterone²².

As it is impossible to cover the entire experimental space that may lead to different solid forms computational methods ensuring that the most relevant forms have been found are in high demand. As yet, several multi-disciplinary solid form screening studies have been performed successfully, complementing experimental screens with crystal structure prediction (CSP) studies.^{4,5,16,32,33} These studies provide a deeper understanding of the solid form landscape of each compound at the molecular level. Computationally generated crystal energy landscapes of creatine³⁴ and carbamazepine²¹ even directed experimental investigations to the finding of new polymorphs.

However, many fine chemicals, in particular pharmaceuticals, exist in both hydrated and anhydrous forms. The stability and behaviour of hydrates can vary significantly,^{35,36} and the transformation routes between hydrate and anhydrate forms have to be studied extensively^{37–44} in order to avoid problems during manufacturing and storage and to ensure the high quality standards of drug products. The dehydration and rehydration processes of a hydrate forming system can be rather complex^{37,41,43}, in particular when the compound forms multiple phases. A hydrate can be the most physically stable form under relevant production and storage conditions whereby the vapour pressure of water in the ambience (relative humidity, RH) is a main key parameter beside temperature and pressure. It is well known that solvent adducts, including hydrates, often crystallise more easily than solvent-free forms, because water/small solvent molecules, as the hydrogen bonding sites of an organic molecule are frequently better satisfied in the presence of a water molecule, which often compensates the lack of hydrogen bond donors or acceptors of a molecular compound.⁴⁵ However, the formation and stability of hydrates is still somewhat unpredictable, despite increasing computational efforts in this field, including CSP.

In seeking to improve our understanding of hydrate formation phenomena in organic (drug) compounds we are expanding our set of organic model hydrate systems^{34,42,46–48} with 4-aminoquinoline (4-amino-2-methylquinoline, **4-AQ**, Fig. 1). We previously investigated the solid form landscape of this compound and its intriguing structural features, ranging from void structure with and without solvent to closed packed structures.⁴⁹ The experimental screen had resulted in three polymorphs (**AH I**^o, **II** and **III**), the monohydrate **Hy1_A** (Cambridge Structural Database⁵⁰ Refcode: LOBSOL51) and a carbon tetrachloride solvate. The computationally generated anhydrate crystal energy landscape allowed us to unravel the complex interplay between close packing and number of strong (hydrogen bonding) interactions in the solid state of **4-AQ**. This was found to be in contrast to the known monohydrate (**Hy1_A**), which adopts a dense crystal packing arrangement and exhibits the maximum number of strong hydrogen bonding interactions.

In the present study we complement the **4-AQ** solid state picture⁴⁹ with the computationally generated monohydrate crystal energy landscape. Surprisingly, the crystal energy landscape indicates that **Hy1_A** is not the global energy minimum hydrate but another monohydrate, which was not observed in the polymorph screen. Thus, we decided to expand our previous state-of-the-art manual polymorphism screen. Guided by the CSP results we not only found the thermodynamically most stable monohydrate polymorph, but also developed a consistent picture of the structural, kinetic and thermodynamic features and inter-relationships of **4-AQ** anhydrides and monohydrates. This recognition required a broad range of analytical techniques, including thermal analysis, X-ray diffraction, gravimetric moisture sorption/desorption studies, spectroscopic methods, water activity and solubility measurements, in combination with computational chemistry.

2 Experimental Section

2.1 Computational Generation of the Monohydrate Crystal Energy Landscape

The planar conformational energy minimum of **4-AQ** was used in the CSP searches. 300,000 $Z'=1$ monohydrate structures were generated using CrystalPredictor^{2.052–54} in 48 common space groups for organic molecules (ESI† Section 1.1). The molecules were held rigid and the lattice energy was evaluated by an exp-6 potential with atomic charges derived using the CHELPG scheme⁵⁵ and minimized. All crystallographically distinct low energy crystal structures were used as starting points for optimising the intermolecular lattice energy (U_{inter}), with an improved model for the intermolecular forces. This was calculated using the FIT56 exp-6 potential parameters and the distributed m+multipoles⁵⁷ derived from the PBE0/6-31G(d,p) charge density using GDMA^{2.58}

The optimal proton positions of the amino group (i.e. pyramidal, deviation from planarity) and methyl group, in all crystal structures within 15 kJ mol⁻¹ of the global minimum (40 structures), were determined using the CrystalOptimizer database method.⁵⁹ This was done by minimising the lattice energy (E_{latt}), calculated as the sum of the intermolecular contribution (U_{inter}) and the conformational energy penalty paid for distortion of the molecular geometry to improve the hydrogen bonding geometries. Conformational energy penalties (E_{intra} , with respect to the pyramidal global conformational energy minimum) and isolated molecule charge densities were computed at the PBE0/6-31G(d,p) level, for

each conformation considered in the minimisation of E_{latt} . All isolated-molecule wave function calculations were performed using GAUSSIAN0960 and intermolecular lattice energies using DMACRYS.61

The 12 most stable structures (within 12 kJ mol⁻¹ of the global minimum) were used as starting points for periodic electronic structure calculations. The DFT-D calculations were carried out with the CASTEP plane wave code62 using the Perdew-Burke-Ernzerhof (PBE) generalized gradient approximation (GGA) exchange-correlation density functional63 and ultrasoft pseudopotentials,64 with the addition of a semi-empirical dispersion correction, either the Tkatchenko and Scheffler (TS)65 or Grimme06 (G06)66 model. For more details see ESI† Section 1.2.

PIXEL calculations67–69 were also performed on the 12 lowest energy structures to estimate the repulsive (E_{R}), dispersion (E_{D}), electrostatic (Coulombic, E_{C}) and polarisation (also called induction, E_{P}) contributions from individual pairs of molecules within a crystal. The charge density for the crystal was constructed from the MP2/6-31G(d,p) ab initio charge density of the isolated molecule as extracted from the computed PBE-TS crystal structures. The electron density was described using medium cube settings and a step size of 0.08 Å, with the pixels condensed into superpixels with a condensation level n=4.

2.2 Materials and Solid Form Screen

Two commercial **4-AQ** samples were used for the investigations, purchased from Aldrich (Lot# STBD1705V, purity 98%) and Fluka (Lot# 248847, purity 98%). The samples consist of **AH I**[°] (Aldrich) and **Hy1A** (Fluka). The 29 solvents used for the polymorphism screen were all of analytical quality and all organic solvents were purchased from Aldrich or Fluka.

The solid form screen repeated the experiments presented in ref. 49 using as educts the commercial samples without purification. Solvent evaporation, cooling crystallisation, anti-solvent addition and liquid assisted grinding experiments were employed for the solvent screening and are detailed in ESI† Sections 2.2 and 2.3.

2.3 Powder X-ray Diffraction

PXRD patterns were obtained using an X'Pert PRO diffractometer (PANalytical, Almelo, NL) equipped with a theta/theta coupled goniometer in transmission geometry, programmable XYZ stage with well plate holder, Cu-K $\alpha_{1,2}$ radiation source with a focussing mirror, a 0.5° divergence slit, a 0.02° Soller slit collimator on the incident beam side, a 2 mm antiscattering slit, a 0.02° Soller slit collimator on the diffracted beam side 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 80s per step in the 2θ range between 2° and 40°. For non-ambient RH measurements a VGI stage (VGI 2000M, Middlesex, UK) was used.

Powder X-ray diffraction data, used for determining the **Hy1B**[°] structure, were collected on a STOE STADI MP diffractometer using strictly monochromatic Cu-K α_1 radiation ($\lambda = 1.54056$ Å) from a focusing Ge(111) primary beam monochromator and a Mythen1k detector with 11° detection range. Measurements were taken in bisecting transmission

geometry at ambient conditions, with a sample of 2 mm diameter placed between two zero-scattering foils. Data were collected in the range from 5 to 120° 2 θ with a step size of 0.009°.

The model was refined, using the computationally generated global minimum structure as starting point, by the Rietveld method employing the FULLPROF.2000 program⁷⁰ and soft constraints. The background was modeled by a set of consecutive points with refineable intensities. The final refinement included a total of 61 parameters (16 profile, 4 cell, 1 scale, 1 isotropic temperature factor, 39 (non H) positions (using 24 soft distance constraints, 13 soft angle constraints) yielding a final R_{wp} = 5.84 (Fig. 2). For further details see §.

The resulting structure from the Rietveld refinement was further scrutinized by allowing all fractional coordinates to refine freely (61 parameters, R_{wp} = 4.64). As expected, the improvement in reliability factors came at the expense of some chemical sense (e.g. bond lengths), but otherwise, the geometry of the molecule was well preserved, confirming the correctness of restrained refined crystal structure.[§]

2.4 Thermal Analysis

For hot-stage thermomicroscopic (HSM) investigations a Reichert Thermovar polarisation microscope, equipped with a Kofler hot-stage (Reichert, A), was used. Photographs were taken with an Olympus DP71 digital camera (Olympus, D).

Differential Scanning Calorimetry (DSC) thermograms were recorded on a DSC 7 (Perkin-Elmer Norwalk, Ct., USA) controlled by the Pyris 2.0 software. Using a UM3 ultramicrobalance (Mettler, Greifensee, Switzerland), samples of approximately 2 - 20 mg were weighed into perforated or sealed aluminium pans or high pressure capsules. The samples were heated using rates in between 2 and 20 °C min⁻¹ and cooled using rates in between 0.1 to 5 °C min⁻¹ with dry nitrogen as the purge gas (purge: 20 mL min⁻¹). The instrument was 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 2-5 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). A heating rate of 5 °C min⁻¹ was applied and dry nitrogen was used as a purge gas (sample purge: 20 mL min⁻¹, balance purge: 40 mL min⁻¹).

[§]Crystal data of 4-aminoquinaldine monohydrate **Hy1B**[°]: C₁₀H₁₀N₂ · H₂O, M_r = 176.2, monoclinic, space group $P2_1/c$, Z = 1, T = 293 K, sample formulation: powder, wavelength: 1.54056 Å, a = 4.7832(5) Å, b = 12.0775(4) Å, c = 16.3371(5) Å, β = 92.226(3), Volume = 943.067 Å³, Z = 4, density = 1.24 g cm⁻³, 2 theta range for data collection: 5-120°, background treatment: 17 background points, No. of measured reflections: 1824, Refinement method: Rietveld with soft constraints, Goodness of fit: 3.7 (on Y_{obs}), R_{wp} = 5.82, R_{exp} = 3.01, R_p = 4.29.

2.5 Karl Fischer Titration

The water content in **4-AQ** samples was determined using a Karl Fischer coulometric titrator C20 instrument (Mettler Toledo, CH).

2.6 Gravimetric Moisture Sorption/Desorption Experiments

Moisture sorption and desorption studies were performed with the automatic multisample gravimetric moisture sorption analyser SPS23-10 μ (ProUmid, Ulm, D). Approximately 150 - 200 mg of sample was used for each analysis. The measurement cycles were started at 40% with an initial stepwise desorption (decreasing humidity) to 0%, followed by a sorption cycle (increasing humidity) up to 90% and back to 0% relative humidity (RH). RH changes were set to 10% for all cycles. Between 0 and 10% RH an additional step at 5% RH was added. The equilibrium condition for each step was set to a mass constancy of ± 0.001 % over 60 minutes and a maximum time limit of 48 hours per step.

2.7 Water Activity Measurements

Excess of **4-AQ AH I**⁴⁹ was stirred (500 r.p.m.) in 1.5 – 2.5 mL of each methanol and water mixture (each containing a different mole fraction of water corresponding to a defined water activity^{45,46} (ESI[†] Section 2.5) at 25.0 ± 0.1 °C for 14 days. Samples were withdrawn, filtered and the resulting phase was determined using PXRD and TGA.

2.8 Determination of Solubility

The Crystal16 crystallisation system (Avantium, NL) was used to determine the kinetic solubilities of **Hy1_A** and **Hy1_B**[°] in water. The temperature at the point the suspension becomes a clear solution upon heating or “clear point” (at 0.1 °C per minute) was taken as the saturation temperature of the measured sample with known concentration. To make sure that solvent-mediated transformations had not occurred during the measurements excess solid was stirred under the same conditions and PXRD patterns of the residual solid were recorded after reaching the highest clear point temperature derived from the solubility experiments.

2.9 Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS system consisted of a HP7890 GC device with a HP5975C inert XL mass-selective detector (Agilent Technologies, Palo Alto, CA, USA). A DB-XLB column (30 m x 0.25 mm i.d. x 0.25 μ m film thickness, J&W Scientific, Folsom, CA) was used for chromatographic separation. Carrier gas was helium with a flow rate of 1.0 mL min⁻¹. 1.0 μ g mL⁻¹ solutions of *SA* and *F* in methanol were used as samples. Injection volume was 2 μ L (splitless), injection temperature was 250 °C. The temperature program was as follows: 50 °C, hold 1 min; increase to 150 °C with 25 °C min⁻¹, to 320 °C with 10 °C min⁻¹, hold for 8 min and to 330 °C in 20 °C min⁻¹, hold for 7.5 min. MS was done in electron impact mode (70 eV) scanning from 50 to 800. Mass spectral data were recorded on a personal computer with the HP MS ChemStation software G1034C version D01.00 (Agilent Technologies).

3 Results

3.1 Computational Screening for 4-AQ Monohydrates

The computationally generated **4-AQ** monohydrate crystal energy landscape (Fig. 3) has several thermodynamically feasible structures within the energy range expected for polymorphic forms.⁷¹ Three structures were found within three kJ mol⁻¹ of the global monohydrate minimum and six within the ten kJ mol⁻¹ range. The structure of the known monohydrate (LOBSOL,⁵¹ **Hy1_A**) corresponds to the second most stable structure in Fig. 3 and was estimated to be 0.7 kJ mol⁻¹ less stable than the PBE-G06 global minimum. The computational results suggest that **Hy1_A** may not be the only **4-AQ** monohydrate and that a more stable hydrate phase could exist. Thus, the previously performed experimental solid form screen⁴⁹ may not have been exhaustive enough, and did not include conditions, which facilitate the formation of one or more further hydrate(s). We therefore started with analysing the computed lowest-energy structures in more depth, contrasting the packings and hydrogen bonding motifs to the experimental forms and expanding the experimental search space for **4-AQ** solid forms (Section 3.2).

The most stable computed monohydrate structures are all densely packed, the packing indices range 70.8% to 74.8%. All **4-AQ** molecules are essentially planar, with the amino group adopting a (slightly) pyramidal conformation. The polar protons deviate from the plane formed by the aromatic ring system optimising the geometry of the hydrogen bonding interactions. In all of the most stable computationally generated hydrate structures (Fig. 3), except structure 8, the maximal number of possible strong hydrogen bonds are formed. All four hydrogen bonding donor groups are used, forming N–H···O, N–H···N, O–H···N or O–H···O interactions (Fig. 4). The amino protons were found to interact either with the water oxygen atom or with the amino nitrogen atom (N2, Fig. 1). One water proton hydrogen bonds to the pyridine nitrogen (N1) and the second to either the amino or the water oxygen atom. The eight structures schematically depicted in Fig. 4 form tetrameric ring motifs with the graph set⁷³ notations: $R_4^2(8)$, $R_4^4(8)$ and $R_4^3(8)$. The ring motifs form one dimensional chains propagating in a zig-zag fashion (Fig. 4e) along the shortest cell axis (ESI† Table S2). The O–H···N1 hydrogen bonds link the molecules into three-dimensional network structures. In addition to the strong hydrogen bonds also $\pi\cdots\pi$ stacking of the aromatic rings was found to contribute significantly to the stability of the eight structures (ESI† Section 1.5).

The hydrogen bonding motifs shown in Fig. 4a & b, as well as Fig. 4c & d, differ solely in the position/connectivity of proton atoms. Structures **Hy1_A** and 3 were found to be isostructural apart from hydrogen atom positions. Thus, the two structures might either “coexist” as a proton disorder structure or due to energetic preference structure 3 might convert to **Hy1_A** through proton transfer. The latter appears more likely as the **Hy1_A** structure solution gave no evidence for proton disorder.

The lowest energy structure forming only three strong hydrogen bonds is structure 8 and was calculated to be 11.5 kJ mol⁻¹ less stable than the global minimum. The global minimum structure is denser packed, more stable (at 0K) and structurally distinct from the known

monohydrate. Therefore this structure must be regarded as an alternative monohydrate polymorph that is likely to be producible experimentally. The predicted structure 8 lacks similarity to the packing arrangements of the other hydrates, and since the calculated energy is high, we assumed that it is rather unlikely to produce this phase experimentally.

3.2 Extended Experimental Screen for 4-AQ (Mono)Hydrate Polymorphs

3.2.1 Moisture Dependent Hydration Studies—The initial experimental screening, 49 performed with purified **4-AQ**, was extended with gravimetric moisture sorption/desorption studies using different **4-AQ** batches as obtained from the supplier and the purified material. This included an over 20 years old sample (Fluka, **Hy1_A**, purity ~98%) and a second, recently purchased sample (Sigma Aldrich, **AH I^o**, purity ~98%). The Fluka sample (*F*, hereafter) had been stored under ambient conditions in a light proofed container and was yellowish in colour, whereas the Sigma Aldrich sample (*SA*) was a white powder, with a similar appearance as batches of **4-AQ** that have been purified by solvent crystallisation (ESI† Section 2.1.).

Moisture sorption/desorption studies using the *SA* and *F* samples are given in Fig. 5a&b. The equilibrium isotherms show distinct steps with plateaus and also a marked hysteresis between the sorption and the desorption cycle, which is a clear indication for a pronounced structural difference between the hydrated and anhydrous phase(s) and for the categorisation of **4-AQ** as stoichiometric hydrate system. The sorption isotherms of the two investigated samples show that **AH I^o** is stable (no water uptake) at and below 40% RH. At moisture conditions above 40% RH the samples take up water forming a hydrate with one mole water per mole **4-AQ**. Above 40% RH the *SA* sample as well as its recrystallisation product (moisture sorption isotherm not shown) resulted in phase pure **Hy1_A**, whereas for the *F* sample a mixture of **Hy1_A** and a second phase was identified with PXRD and IR spectroscopy. The new phase was expected to be a second monohydrate polymorph, finally named **Hy1_B^{°§§}**.

The desorption cycles show that the transformation of the monohydrate(s) to **AH I^o** occurs only at very dry conditions (5% RH and below), indicating that the two hydrates are stable under conventional storage conditions. In contrast **AH I^o** (thermodynamically most stable anhydrate polymorph in the entire temperature range⁴⁹) transforms to the monohydrate(s) already under usual indoor climate conditions (25°C, 40 – 60 % RH), suggesting that the hydrated forms should be used if (some) moisture cannot be avoided.

The structural changes upon hydration were monitored with moisture controlled PXRD experiments (Fig. 5C, D). To minimise particle size depending effects on hydration kinetics the samples were sieved (150 µm mesh). The two **AH I^o** samples did not show any changes upon increasing the RH from 0 to 40% RH (Fig. 5C, D). On exceeding this moisture condition sample *SA* Transforms to **Hy1_A** whereas sample *F* shows reflections of **Hy1_A** and the second monohydrate **Hy1_B^o**.

§§The thermodynamically stable form at room temperature is flagged with the symbol “°”.

3.2.2 Solvent Screen using Different 4-AQ Batches/Purities—Driven by the finding of the new **Hy1_B**[°] We repeated the experimental screen for solid forms⁴⁹ using the two **4-AQ** batches (*SA* and *F*) as obtained from the supplier.

Evaporation, cooling crystallisation, precipitation or liquid assisted grinding experiments with the original (un-purified) *SA* sample (ESI† Section 2.2) did not result in other forms than those observed in a previous screening programme.⁴⁹ This is also true for the *F* sample except for a few crystallisations, which resulted in a mixture of **Hy1_A** and **Hy1_B**[°] (ESI† Tables S9): fast and slow cooling crystallisation experiments from n-propanol or methyl acetate, whereas slow crystallisation experiments led to higher proportions of the new phase compared to fast crystallisation. **Hy1_B**[°] was also formed in slow cooling crystallisation experiments from acetonitrile or dichloromethane, concomitantly with **Hy1_A** and **AH I**[°]. TGA and coulometric Karl Fischer titration experiments of the n-propanol or methyl acetate samples indicated, despite being a mixture, the stoichiometry of a monohydrate. Differences in optical appearance of the purified or original *SA* (white powder) and *F* batches (yellowish powder) as well as the fact that the new phase was exclusively obtained with the *F* sample suggested that the impurity profiles of *SA* and *F* must be different and have an influence on the crystallisation/nucleation process. GC-MS (Fig. 6) and ESI-MS (ESI† Section 2.1.2) confirmed that the *SA* and *F* samples contain different chemical impurities in various quantities. Two **4-AQ** derivatives could be identified as impurities: impurity 1 was identified as chloro-substituted **4-AQ** ($M_r = 192$) and impurity 2 is presumably a carboxaldehyde-derivative of **4-AQ** or N-(2-methyl-4-quinoliny)-formamide ($M_r = 186$). For more information see ESI† Section 2.1. Further investigations focussing on the impact of the identified impurities on the nucleation of **4-AQ** forms are planned.

3.2.3 Crystallisation under Hydrostatic Conditions—CSP indicated that **Hy1_B**[°] is the most stable monohydrate structure at 0K with the highest packing index (Fig. 3). This result suggests that elevated pressure may favour the formation of this hydrate. To verify this suggestion, **AH I**[°] (sample *SA*) was suspended in an excess of water (about 15 mg in roughly 7 mg of water) and heated to 125 °C in an hermetically sealed (high pressure) DSC pan. Subsequently the sample was slowly cooled to 25 °C (0.1 K min⁻¹, in the sealed pan) and the phase identity was tested with PXRD. This hydrothermal method resulted in **Hy1_B**[°] confirming our suggestion. The crystallisation of the hydrate on cooling occurred in the temperature range between 82 and 86 °C. Increasing the water ratio lowers the crystallisation temperature range (77 to 82 °C) and more importantly results in the metastable hydrate **Hy1_A**. Thus, it is obvious that pressure favours the formation of the stable monohydrate **Hy1_B**[°] besides certain impurities which obviously induce the nucleation of this hydrate during the hydration process of the anhydrate at elevated moisture conditions.

3.3 Crystal Structures of the Monohydrates

The structure of the metastable **Hy1_A**, determined by Tai et al. (LOBSOL),⁵¹ is contrasted to the new polymorph of this monohydrate which was solved from laboratory PXRD data.

3.3.1 Monohydrate Hy1_A—The hydrate crystallises in the orthorhombic space group *Pna*2₁ with each one **4-AQ** and one water molecule in the asymmetric unit. The planar **4-AQ**

molecule forms three hydrogen bonding interactions (Fig. 4a), one $\text{N2-H1}\cdots\text{N2}$, involving an adjacent **4-AQ** molecule, and two $\text{4-AQ}\cdots\text{water}$ interactions ($\text{N2-H2}\cdots\text{Ow}$ and $\text{Ow-H}\cdots\text{N1}$, respectively). A fourth strong H-bond is formed between water molecules, leading to a chain of water molecules propagating along [100]. A further characteristic of the structure are $\pi\cdots\pi$ stacks and $\text{C-H}\cdots\pi$ interactions of **4-AQ** molecules. The water molecules are located in channels surrounded by the polar groups of the **4-AQ** molecule and show a tetragonal coordination (Fig. 7). Assumable, the dehydration mechanism starts with the removal of the water through the [100] channels (ESI† Section 1.6).

3.3.2 Monohydrate Hy1_B[°]—The second **4-AQ** monohydrate structure has $P2_1/c$ space group symmetry, with one **4-AQ** and one water molecule in the asymmetric unit. All four hydrogen bonding donor groups are used and form four strong $\text{4-AQ}\cdots\text{water}$ intermolecular interactions, i.e. $\text{N2-H1/2}\cdots\text{Ow}$ and $\text{Ow-H}\cdots\text{N1/2}$ (Fig. 8). As in **Hy1_A**, the **Hy1_B[°]** structure forms tetrameric hydrogen bonded ring motifs, arranged in zig-zag fashion along the crystallographic [100] axis (Fig. 4a) involving the amino groups and water molecules. The water molecules in the structure of **Hy1_B[°]** are strongly bound, tetragonally coordinated (alike in **Hy1_A**) but are isolated from each other and thus lack strong water $\cdots$ water hydrogen bonding interactions. Similar as in **Hy1_A**, the **Hy1_B[°]** packing exhibits polar and apolar regions and a channel-like arrangement of the water molecules in direction of the crystallographic [100] axis. Both structures are three-dimensional network structures. Dehydration of **Hy1_B[°]** may also be expected to start with the removal of the water through the channels, along the crystallographic [100] axis.

The two monohydrate structures share the same type of 1D stacked **4-AQ** molecules, i.e. $\pi\cdots\pi$ stacks along [100]. However, the two polymorphs differ substantially in hydrogen bonding interactions (involved groups, Fig. 4) and the overall packing arrangement (ESI† Section 2.7). Thus, a transformation between the two hydrates requires breaking of strong intermolecular interactions (see Section 4.1) and a considerable rearrangement of the molecules explaining the high kinetic stability of the metastable monohydrate **Hy1_A** and the fact that a transformation of this hydrate to the stable monohydrate polymorph **Hy1_B[°]** was not observed in slurry experiments (see Section 3.4).

3.4 Thermodynamic and Kinetic Stability of the 4-AQ Monohydrates and AH I[°]

3.4.1 RH Stability—In order to overcome the kinetic control governing the gravimetric moisture sorption/desorption studies, the water activity where the anhydrate transforms to a hydrate was determined in methanol/water mixtures. Samples of **4-AQ AH I[°]** (recrystallised from ethanol and dried at 100 °C) were added to methanol/water mixtures of various compositions (ESI† Section 2.5) representing different water activities (a_w : 0 to 1) and the suspensions were stirred for two weeks at 25 °C to reach an equilibrium state. At a water activity (a_w) < 0.15, the isolated solid phase contained only **AH I[°]** (Fig. 9) whereas at a_w 0.15 **Hy1_A** was identified, suggesting that the equilibrium between the anhydrate and the monohydrate **Hy1_A** lies at an a_w of ~ 0.14 at 25 °C. Repeating the water activity slurry experiments using the dried *F* sample resulted in the same thermodynamic stability ranges for anhydrous and hydrated **4-AQ**.

The results of this a_w study illustrates that the thermodynamic equilibrium between a hydrate and an anhydrate may be situated on one side of the hysteresis range observed in the moisture sorption/desorption experiments and not in the centre (see Fig. 5a,b). This suggests that the kinetic mechanisms and activation barriers of the two reversible processes (hydration $\leftrightarrow$ dehydration) may vary considerably. The kinetic control of the anhydrate (**AH I** $^\circ$) to hydrate (**Hy1_A**) transformation (hydration) is significantly stronger than in the reversible process (dehydration). This is indicated by the fact that the hydration occurs at an atmospheric moisture condition of $a_w \approx 0.5$ (50% RH) and thus far above the true equilibrium $a_w = 0.14$ ($a_w \approx 0.3$). In contrast, dehydration of **Hy1_A** occurs at an atmospheric moisture condition of $a_w \approx 0.05$, which is very close to the equilibrium state ($a_w \approx 0.1$) and indicates a distinctly weaker kinetic control for this dehydration process compared to hydration.

Hy1_B $^\circ$ was neither formed in any of the methanol/water slurries nor in liquid assisted grinding experiments, independent whether **AH I** $^\circ$, **Hy1_A** or a mixture of these forms with **Hy1_B** $^\circ$ was used as starting material (time frame: two weeks). Since both hydrates were stable in any test involving solvents of sufficiently high water activity ($a_w > 0.14$) it was not possible to establish the thermodynamic stability order of the two polymorphic monohydrates based on the performed experiments.

3.4.2 Thermal Stability—Since we failed to transform one of the polymorphic monohydrates in slurry experiments and by liquid assisted grinding we decided to determine the order of their thermodynamic stability via temperature dependent solubility experiments using a Crystal16 parallel reactor system and pure water as the solvent. As shown in Fig. 10 the water solubility of **Hy1_B** $^\circ$ is lower than that of **Hy1_A** which suggests that **Hy1_B** $^\circ$ is the thermodynamically stable **4-AQ** monohydrate in the investigated temperature range (30 to 70 °C).

Based on the extrapolated intersection of the van't Hoff plots (Fig. 10b) the transition point between the two hydrate phases lies around 119 °C, which is only a very rough figure as such an extrapolation far beyond the experimental range is very error-prone. As shown later, the extrapolated transition point lies above the melting points of the two hydrates, suggesting a monotropic relationship. Therefore, the solubility data suggest that **Hy1_B** $^\circ$ is also the thermodynamically stable hydrate below RT (down to 0K), which is in agreement with the computational results (Fig. 3). Thermodynamic parameters, the enthalpy of solution (H_{sol}) and entropy of solution (S_{sol}) were estimated from the slope and constant of the regression line of the van't Hoff plot, respectively (Table 1). The enthalpies and entropies of solution are positive for the two hydrates and greater for **Hy1_B** $^\circ$.

Based on the solubility difference of the two hydrate polymorphs shown in Fig. 10 we were able to produce **Hy1_B** $^\circ$ from **Hy1_A/Hy1_B** $^\circ$ mixtures. The fact that not only water, but also organic solvents such as n-propanol or methyl acetate preferentially dissolved **Hy1_A** indicates that also at lower a_w **Hy1_B** $^\circ$ is more stable than **Hy1_A**.

In addition to the solubility experiments HSM, TGA and DSC were employed to determine the thermal stability of the hydrates. Dehydration of **Hy1_B** $^\circ$ started at 40 °C and dehydration

of **Hy1_A** at 60 °C (Fig. 11, TGA and “open” DSC runs). The hydrate crystals maintain their original shape but split into polycrystalline aggregates (exemplary shown for **Hy1_A** in Fig. 11, photomicrographs). This behaviour, termed ‘pseudomorphosis’,⁷⁴ is characteristic for stoichiometric solvates and is indicative for a pronounced reorganisation of the structure upon desolvation. Thermal dehydration of the two hydrate phases resulted exclusively in **AH I°**. Upon further heating sublimation of **AH I°** starts at a temperature above 130 °C in agreement with previous observations.⁴⁹ It has to be noted that the **Hy1_B°** batch consisted of very small crystals (powder) whereas the used **Hy1_A** sample contains larger crystals. Therefore, the onset temperature of the dehydration reaction does not allow conclusions about the thermal stability order of the two polymorphs, as crystal size influences the onset temperature of dehydration.⁷⁵

The TGA curves of the pure hydrates show a one-step mass loss. The mass loss observed in the TGA experiments corresponds for each of the hydrates to exactly one mol of water per mol **4-AQ**.

The DSC measurements of **Hy1_A** and **Hy1_B°** were carried out either in pin-holed or sealed pans and at different heating Structures showing low packing efficiency and an unused hydrogen bond donor rates to investigate the influence of atmospheric conditions on the dehydration reaction. A broad desolvation endotherm is observed when samples are heated in pans with a five pin holed lid (Fig. 11, DSC open). The heats of dehydration were determined to be $53.5 \pm 0.3 \text{ kJ mol}^{-1}$ ($_{\text{dehy}}H_{\text{Hy1A-I}^\circ}$, $T_{\text{max}} = 95 \text{ }^\circ\text{C}$) and $57.1 \pm 0.2 \text{ kJ mol}^{-1}$ ($_{\text{dehy}}H_{\text{Hy1B-I}^\circ}$, $T_{\text{max}} = 70 \text{ }^\circ\text{C}$) for the **Hy1_A** and **Hy1_B°** to **AH I°** transformations, respectively. Congruent melting of the hydrate crystals can be determined at $111.1 \pm 0.2 \text{ }^\circ\text{C}$ for **Hy1_A** and $113.1 \pm 0.2 \text{ }^\circ\text{C}$ for **Hy1_B°**, if the hydrate sample is heated in sealed DSC pans or embedded in high viscosity silicon oil in hot-stage microscopic investigations. Based on the measured melting temperatures and $_{\text{dehy}}H$ enthalpies it can be concluded that the two hydrate forms are monotropically related, with **Hy1_B°** being the thermodynamically stable hydrate phase in the entire temperature range. **Hy1_B°** shows a higher melting temperature and higher enthalpy of dehydration to **AH I°** compared to **Hy1_A**. The DSC results are in agreement with the hydrate solubilities measured in water.

3.4.3 Enthalpy of Hydrate/Anhydrate and Hydrate/Hydrate Transformations—

The enthalpies of the hydrate to anhydrate phase transformations ($_{\text{trs}}H_{\text{Hy1-I}^\circ}$) were estimated by subtracting the heat of vaporisation of one mol of water, $_{\text{vap}}H$, from $_{\text{dehy}}H_{\text{Hy1-I}^\circ}$ at the dehydration temperature⁷⁶ (eq.1).

$$\Delta_{\text{tr}}H_{\text{Hy1-I}^\circ} = \Delta_{\text{dehy}}H_{\text{Hy1-I}^\circ} - \Delta_{\text{vap}}H_{\text{H}_2\text{O}} \quad (1)$$

This resulted in $_{\text{trs}}H_{\text{Hy1A-I}^\circ}$ of $11.9 \pm 0.2 \text{ kJ mol}^{-1}$ for the **Hy1_A** to **AH I°** transformation and $_{\text{trs}}H_{\text{Hy1B-I}^\circ}$ of $15.4 \pm 0.3 \text{ kJ mol}^{-1}$ for the **Hy1_B°** to **AH I°** transformation, using an average $_{\text{vap}}H_{\text{H}_2\text{O}}$ value of $41.858 \text{ kJ mol}^{-1}$ (80 °C). The **Hy1_A** phase was estimated to be $3.6 \pm 0.4 \text{ kJ mol}^{-1}$ less stable than **Hy1_B°** (Table 1), in agreement with the stability order derived from the solubility data.

By comparing the lattice energies of the hydrates, the dehydration product **AH I**[°] and ice (59 kJ mol⁻¹)^{77,78} a simple estimation of $\Delta_{dehy}U_{Hy-AH}$ and $\Delta_{trs}U_{Hy-AH}$ can be made according to eqs. (2) and (3).

$$\Delta_{dehy}U_{Hy1-I^{\circ}} = -E_{latt(Hy)} - (-E_{latt(AH)}) \quad (2)$$

$$\Delta_{trs}U_{Hy1-I^{\circ}} \approx \Delta_{dehy}U_{Hy1-I^{\circ}} - n(-E_{latt(ice)}) \quad (3)$$

$\Delta_{trs}U_{Hy1A-I^{\circ}}$ was calculated to be 20.95 kJ mol⁻¹ for the **Hy1_A** to **AH I**[°] and 21.62 kJ mol⁻¹ for the **Hy1_B**[°] to **I**[°] phase transformation. These results not only confirm that hydrate formation is driven by a greater potential energy of the hydrates but also that the monohydrates are stable phases (phase transition values of ca. 20 kJ mol⁻¹).

The energy differences of 0.7 kJ to 4.0 kJ mol⁻¹ (Table 1) between the monohydrate polymorphs, derived from static 0K lattice energy calculations, are of comparable magnitude to the experimentally measured data. Thus, the computational method not only correctly predicted the structures but also gave a reasonable energy estimation of the hydrate/anhydrate and hydrate/hydrate transformation energies.

3.4.4 Hy1_A to Hy1_B[°] Phase Transformation—The experiments presented in Section 3.4.1 were complemented with long term storage experiments to investigate the **Hy1_A** to **Hy1_B**[°] phase transformation. Phase pure and mixed hydrate samples were stored at ambient conditions, as well as at higher RH values (61%, 75%, 84% and ~100%), for one year. A slow, but partial phase transformation from **Hy1_A** to **Hy1_B**[°] was observed for mixed hydrate samples stored at RH values in the range between 61% and 75% RH, in agreement with the monotropic relationship of the two hydrate polymorphs. Surprisingly, no change in phase composition was observed if mixtures were stored at high RH values (84% and 100% RH) after one year. At present, we do not have a plausible explanation for this behaviour. The pure hydrate samples did not transform within the investigated time period of one year. The fact that the 20-years old *F* sample did not transform to **Hy1_B**[°] indicates that metastable **Hy1_A** shows a very high kinetic stability.

4 Discussion

4-AQ shows a diverse solid form landscape (Fig. 12) with intriguing structural and stability phenomena. The combination of computational chemistry with experimental screening and solid form characterisation techniques allowed us to unravel the complex kinetic and thermodynamic stability relationship between the hydrates and **AH I**[°]. The results of the study complement and extend the recently established structural and thermodynamic relationships between the anhydrate polymorphs and the carbon tetrachloride solvate of **4-AQ**. We now have achieved a thorough understanding of the solid state behaviour of the small organic drug-like model compound. The novel phase, **Hy1_B**[°], is the

thermodynamically most stable phase and monotropically related to the kinetic **Hy1_A** polymorph. Only at low RH values (< 14% RH) water free **4-AQ**, **AH I°**, becomes more stable than the hydrates. The finding of the most stable phase, **Hy1_B°**, was complicated by the fact that the metastable but kinetically stabilised hydrate nucleates/grows more easily than **Hy1_B°**.

4.1 Why does 4-AQ form Hydrates?

In the anhydrous state **4-AQ** was found to appear to some extent frustrated with respect to its packing efficiency and hydrogen bonding ability. Structures showing low packing efficiency (67.4%) and an unused hydrogen bonding donor group were characteristic of the computed anhydrate crystal energy landscape.⁴⁹ This is in contrast to Fig. 3 which has densely packed and with respect to hydrogen bonding interactions saturated structures (Fig. 4) as lowest energy structures.

The mismatch of hydrogen bond donor and acceptor groups in **AH I°**, only one of the two donor groups is involved in a strong intermolecular interaction, can be overcome by addition of water which acts as a strong hydrogen bonding donor and acceptor in **Hy1_A** and **Hy1_B°**. Pair-wise intermolecular energy calculations (U_{inter}), derived from PIXEL energy calculations (Table 2) showed that the strongest interactions in the hydrate structures are formed between the water and **4-AQ** molecules. The water...**4-AQ** interactions per se are weaker than the strongest **4-AQ**...**4AQ AH I°** hydrogen bond, however, this is compensated for by the fact that the water molecule forms four of the strongest intermolecular interactions in the hydrates (Table 2). The strongest **4-AQ**...**4-AQ** pair-wise interaction observed in a hydrate, **Hy1_A**, was estimated to be less than half of the strength of the **4-AQ**...**4-AQ AH I°** N2–H2...N1 hydrogen bond.

The water molecules in **Hy1_A** and **Hy1_B°** are well accommodated and strongly bound in the framework of the hydrates and account for more than half of the intermolecular energy (U_{inter}). Pairwise **4-AQ**...**4AQ** interactions were estimated to account for 47% and 42% of the **Hy1_A** and **Hy1_B°** U_{inter} , respectively. Water...**4-AQ** interactions for 40% in **Hy1_A** and for 55% in **Hy1_B°**. And finally water...water interactions account for the remaining 13% and 3% in **Hy1_A** and **Hy1_B°**, respectively. This may rationalise why upon dehydration a structural collapse to a distinct crystal packing, **AH I°**, is observed and not the formation of an isostructural dehydrate.⁷⁹ Water egress is likely to occur along the [100] axis in each of the two monohydrates, but this process is accompanied with a disruption of the 3D-network structures. Overall, the formation of additional and strong intermolecular interactions in the hydrate structures seems to be the driving force for hydrate formation.

The PIXEL U_{inter} calculations not only indicate water as the key pillar to the hydrate structure stabilities, but also the kinetic stability of the **AH I°** structure with respect to the stable hydrates. Breaking the strong N2–H2...N1 intermolecular interaction will require an appreciable activation energy. This may indicate why the sorption step in Fig. 5 is shifted to higher RH values (> 40% RH) relative to the estimated critical water activity of ~0.14.

4.2 Value of CSP and Lattice Energy Calculations in Solid Form Screening and Characterisation

The computationally generated monohydrate crystal energy landscape (Fig. 3) unambiguously indicated the possibility of another more stable polymorph, which corresponds to **Hy1_B**. **4-AQ** is a prime example where CSP can be seen as an invaluable tool for revealing whether a more stable phase may exist and affirming whether an experimental screening should be extended or not. Thus, CSP despite being computationally time consuming, in particular for multi-component,⁸⁰ flexible and bigger systems,^{81,82} has to be seen as an alternative and complementary screening approach.

The lattice energy calculations, using different methods, based on ab initio electronic calculations on either the crystal (DFT-D) or single molecule (CrystalOptimizer), solubility and thermal measurements give the same qualitative result that the new hydrate, **Hy1_B**, is more stable than **Hy1_A**. The experimental energy differences between the two hydrates were found to be $3.6 \pm 0.4 \text{ kJ mol}^{-1}$ (Table 1) when calculated from the dehydration enthalpies and $\sim 5.6 \text{ kJ mol}^{-1}$ when derived from Crystal16 solubility experiments. The lattice energy differences give estimates ranging from 0.7 to 4.0 kJ mol^{-1} depending on the applied method (Table 1). The computationally derived energy differences are in good agreement with the experimental values considering that the calculations neglect zero-point and thermal effects. Computing the energy difference between a hydrate and anhydrate is more challenging than between polymorphs. The applied functional, PBE, is well known to overestimate water interactions,⁸³ which may explain why the calculations overestimate the hydrate to anhydrate transition energies (Table 1). The measured and computed energy differences were found to be in the range of other hydrate/anhydrate systems showing a stable hydrate phase, e.g. phloroglucinol dihydrate⁴⁶ and creatine monohydrate.³⁴ Thus, the formation of hydrates, its structures and that the hydrates are stable with respect to the anhydrides could have been predicted.

4.3 Why was **Hy1_B** not Found Earlier?

Firstly, the metastable **Hy1_A** shows a very high kinetic stability as evidenced from long time storage experiments of the *F* sample. Even after 20 years the **Hy1_A** did not transform to the thermodynamically stable **Hy1_B** polymorph. Slurry experiments of **Hy1_A** or mixtures of **Hy1_A/Hy1_B** at RT conditions ($20 - 30 \text{ }^{\circ}\text{C}$ and $a_w > 0.14$) did not show any change in phase composition. Only lengthy storage experiments of a **Hy1_A/Hy1_B** mixture in the RH range in between 61% and 75 % showed, after a year, that **Hy1_A** transforms very slowly to **Hy1_B**. The reason for the slow transformation kinetics can be seen in an high activation energy barrier for the polymorphic phase transformation, as indicated by the different crystal packings, a phase transformation requires considerable rearrangement of the **4-AQ** and water molecules, and the strong pair-wise intermolecular interactions present in **Hy1_A** (PIXEL energy calculations). Thus, phase pure **Hy1_A** is storage stable and can be handled without risk of inducing any phase transformations provided low RH values and higher temperatures are avoided.

Secondly, crystallisation of **Hy1_B** at ambient pressure was found to be tied to a phase impurity, a chloro-4-aminoquinoline derivative with a M_r of 186 (ESI† Section 2.1.3). **Hy1_B**

crystals obtained at the same conditions as **Hy1_A** crystals were smaller, indicating a slower growth rate of **Hy1_B[°]** compared to **Hy1_A** in the solvents that allowed the nucleation of **Hy1_B[°]**. Only specific crystallisations of impure **4-AQ** from n-propanol, methyl acetate, acetonitrile or dichloromethane or exposure of impure anhydrous **4-AQ** to high but not extreme moisture conditions yielded **Hy1_B[°]**. The fact that neither crystallisation, lyophilisation nor slurry experiments using water yielded **Hy1_B[°]** may be indicative that water favours the nucleation/growth of the metastable hydrate. This may be attributed to the fact that in **Hy1_A** direct water···water interactions are observed (as in solution), but water molecules are isolated in **Hy1_B[°]** which would require breaking the water···water interactions if water is incorporated from solution. The impurity is likely to inhibit the nucleation/growth of the kinetic form and thus facilitates the nucleation/growth of the thermodynamic form. Using a purified **4-AQ** batch for the initial polymorph screen prevented us from finding the stable monohydrate form. This highlights the importance of additives as another variable in polymorphism screens as also demonstrated by the steadily growing list of examples where attempts to form co-crystals failed, but instead resulted in new polymorphs of a co-former (e.g., isonicotinamide⁸⁴, gallic acid monohydrates⁸⁵ and brucine dihydrate⁸⁶).

Thirdly, only by applying hydrothermal conditions (pressure) we were able to obtain **Hy1_B[°]** from chemically pure anhydrous **4-AQ**. The variable pressure has been reported to be successful in producing solvates and polymorphic forms.^{87,88} The hydrothermal crystallisation experiments were suggested by the results of CSP (Fig. 3) which indicated a high density structure as the global energy minimum. Thus, the computationally generated crystal energy landscape not only indicated that a more stable **4-AQ** monohydrate should exist but also suggested a possible route for preparing the novel polymorph.

5 Conclusions

4-Aminoquinoline shows both anhydrate and monohydrate polymorphism, with the hydrates being the stable forms under conditions of relative humidity (RH) typically found on production and storage. The metastable hydrate polymorph, **Hy1_A**, shows an appreciable kinetic stability and if phase pure, no conversion to the monotonically related, thermodynamically stable **Hy1_B[°]** polymorph was observed during storing the metastable phase for over 20 years at ambient conditions. Crystal structures and lattice energy calculations rationalise the stability behaviour. The introduction of water molecules generates a hydrogen bonding network of higher dimension as there is a better match between the numbers of hydrogen bond donor and acceptor groups than in the anhydrides, explaining the high tendency towards hydrate formation of **4-AQ**.

It should be stressed that the discovery of the most stable solid form of **4-AQ** in the present study occurred 80 years after its first synthesis.⁸⁹ It is particularly notable that this finding was guided by the computationally generated crystal energy landscape, which indicated the existence of a second more stable monohydrate with a higher density than the known monohydrate form. Crystallising **Hy1_B[°]** was complicated by the fact that the high density form shows a slow nucleation and growth rate, in contrast to **Hy1_A**. Therefore, a comprehensive classical polymorph screen only yielded the metastable monohydrate **Hy1_A**. Thanks to the availability of an impure sample of **4-AQ** the stable monohydrate **Hy1_B[°]**

could be experimentally produced, highlighting the remarkable impact of specific impurities on the nucleation process. Nevertheless, it was still difficult to produce phase pure **Hy1_B**^o because of its very slow crystal growth rate why conventional strategies such as solvent mediated transformation experiments did not result in **Hy1_B**^o. After having identified specific crystallisation conditions (hydrothermal) it was possible to obtain the thermodynamically most stable form in reasonable time.

Hence, this study highlights a serious problem in solid form screening and demonstrates clearly that finding the most stable solid form is not straightforward. Since it is practically impossible to exploit all experimental conditions that may lead to different solid forms⁸ the discovery of specific (say “practically useful”) crystal forms may be very difficult and requires some serendipity and/or high experimental demands. Theoretical methods may not only give assurance that relevant forms have been found, but may also give important indication of the existence of a “hidden” low energy form. The use of CSP as a complementary method to experimental solid form screening is therefore highly beneficial for identifying practical crystal forms. However, more experience is needed to identify the thermodynamically plausible structures and eliminate computed structures that are unlikely to be observed.^{71,90} This is in particular true for more complex (flexible and drug-like), $Z' > 1$ and charged molecules (e.g. salts), for which currently considerable computing time is required and may not give reliable results.

Finally, it should be indicated that creatine is a related case, where the thermodynamically stable anhydrate form has been predicted and experimentally verified (independently by us³⁴ and Arlin et al.⁹¹) 180 years after the discovery of the compound. However, in the case of creatine the formation and high stability of the monohydrate hampered the experimental access to the stable anhydrates.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.
Molecular diagram of 4-amino-2-methylquinoline (4-aminoquinaldine, **4-AQ**) monohydrate.

Fig. 2.

Rietveld refinement from PXRD data. Experimental intensities (points), calculated intensities (line), difference plot (line at the bottom). Green vertical bars indicate the positions of the Bragg peaks.

Fig. 3. Crystal energy landscape for **4-AQ** monohydrates, classified by space group. PI - Kitaigorodskii type of packing index, calculated using PLATON.⁷² Each symbol denotes a crystal structure. Experimental structures are encircled and labelled as **Hy1_A** (LOBSOL51) and **Hy1_B[°]**. Arabic numbers are used for the most stable hypothetical structures and the numbers correspond to their stability rank (ESI† Table S2).

Fig. 4.

Hydrogen bonding motifs observed in the lowest computationally generated monohydrate structures (Fig. 3). Structure numbers correspond to energy rank. Nitrogen atoms are labelled according to Fig. 1.

Fig. 5.

(a,b) Gravimetric moisture sorption and desorption curves of **4-AQ** at 25 °C: (a) starting with sample *SA* and (b) starting with sample *F* (both AH I°). The grey circles represent data points reaching the equilibrium (constant mass) within the pre-set maximal equilibration time (see experimental section), 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) Moisture dependent PXRD measurements showing the hydration process of **4-AQ** sample *SA* (AH I° to Hy1_A) and (d) sample *F* (AH I° to a mixture of Hy1_A and Hy1_B°). The angular range of characteristic reflections of Hy1_A are marked in blue whereas a yellow colour highlights the range of distinct Hy1_B° reflections. Due to different equilibration times and other parameters such as sample amount, dynamics of the atmosphere etc. the hydration kinetics in the gravimetric moisture chamber (SPS) is different to kinetics in the moisture stage (VGI) used for the PXRD recordings.

Fig. 6.
GC-MS impurity screening in 4-AQ samples *SA* and *F*.

Fig. 7. Packing diagram of **Hy1_A**, viewed along the crystallographic [100] axis. Hydrogen bonding interactions are indicated with dotted lines.

Fig. 8. Packing diagram of **Hy1B**[°], viewed along the crystallographic [100] axis. Hydrogen bonding interactions are indicated with dotted lines.

Fig. 9.

Phase diagram of **4-AQ** at different water activities in methanol/water mixtures at 25 °C.

The **AH I°** was used as starting phase, the residual phase, after stirring the suspensions for two weeks, was determined with PXRD.

Fig. 10.

(a) Solubility (mole fraction) of **4-AQ** monohydrates in water ($a_w = 1.0$) as a function of temperature. (b) Van't Hoff plot of the molar solubility as a function of temperature.

Fig. 11.

TGA curves of **Hy1A** and **Hy1B** recorded at a heating rate of 5 °C min⁻¹. DSC thermograms of the two hydrate polymorphs were recorded in five pin-holed pans (open) at a heating rate of 5 °C min⁻¹ and in sealed pans (closed) at a heating rate of 10 °C min⁻¹. T_{fus} – melting point. Photomicrographs show exemplarily the pseudomorphosis of **Hy1A** upon dehydration.

Fig. 12.

Preparation and transition pathways of the **4-AQ** solid forms at different temperatures, relative humidities and hydrothermal conditions. **AH I^o**, **II** and **III** – anhydrous forms, **Hy1_A** and **Hy1_B^o** – monohydrate polymorphs, **S_{CCl4}** – carbon tetrachloride solvate. Superscript “o” indicates the thermodynamically stable anhydrate/monohydrate form.

Table 1
Values of the thermodynamic parameters obtained from solubility and thermal measurements and lattice energy calculations.

	Hy1 _A	Hy1 _B ^o	(Hy1 _A – Hy1 _B ^o)
<i>Solubility Measurements (30 – 70 °C, water)</i>			
H_{sol} , kJ mol ⁻¹ ^a	37.3	42.8	–5.6
S_{sol} , J mol ⁻¹ K ⁻¹ ^b	45.3	59.5	–14.2
<i>Thermal Measurements (55– 100 °C)</i>			
T_{fus} (°C) ^c	111.1 ± 0.2	113.1 ± 0.2	–2.0 ± 0.4
$_{\text{dehy}}H_{\text{Hy1x-I}^{\circ}}$ ^d	53.5 ± 0.3	57.1 ± 0.2	–3.6 ± 0.5
$_{\text{trs}}H_{\text{Hy1x-I}^{\circ}}$ ^e	11.9 ± 0.2	15.4 ± 0.3	–3.6 ± 0.4
<i>Lattice Energy Calculations (0 K)</i>			
$-E_{\text{latt}}$ (kJ mol ⁻¹), CryOpt (PBE0/6-31G(d,p))	172.65	176.66	–4.01
$-E_{\text{latt}}$ (kJ mol ⁻¹), PBE-TS	234.45	232.48	–1.97
$-E_{\text{latt}}$ (kJ mol ⁻¹), PBE-G06	212.13	212.80	–0.73
$_{\text{dehy}}U_{\text{Hy1x-I}^{\circ}}$, ^d PBE- G06	79.95	80.62	
$_{\text{trs}}U_{\text{Hy1x-I}^{\circ}}$, ^e PBE- G06	20.95	21.62	

^aEnthalpy of solution, from the slope of van't Hoff plot (slope = – H_{sol}/R).

^bEntropy of solution, from the constant of the regression line (constant = – S_{sol}/R).

^cMelting point (onset temperature).

^dEnthalpy of dehydration.

^eEnthalpy of hydrate to **AH I**^o phase transformation.

Table 2
PIXEL energy calculations on 4-AQ AH I° and monohydrate structures using the PBE–TS structure models. Only the strongest intermolecular interactions for pairs of molecules are listed.

Phase	Interaction ^a	E_C^b	E_P^c	E_D^d	E_R^e	U_{inter}^f
		kJ mol ⁻¹				
AH I°	N2–H2...N1	–62.5	–29.4	–31.4	76.4	–46.9
	$\pi \cdots \pi$	–0.2	–3.7	–32.5	19.0	–17.4
	C–H... π	–9.7	–4.8	–19.7	17.6	–16.6
Hy1 _A	Ow–H...N1	–87.6	–49.7	–22.8	133.2	–27.0
	N2–H1...Ow	–41.9	–14.4	–9.3	40.9	–24.7
	N2–H2...N2 and C–H... π	–16.9	–9.3	–27.7	31.2	–22.7
	$\pi \cdots \pi$	–2.7	–5.8	–43.0	31.6	–19.9
	Ow–H...Ow	–59.3	–25.4	–8.6	77.0	–16.4
Hy1 _B	N2–H1...Ow	–36.0	–11.5	–10.1	30.1	–27.4
	N2–H2...Ow	–43.9	–16.5	–12.4	48.9	–24.0
	$\pi \cdots \pi$	–5.6	–5.7	–41.8	30.3	–22.9
	Ow–H...N1	–87.6	–52.6	–21.4	138.9	–22.6
	Ow–H...N2	–36.6	–18.5	–11.9	49.0	–18.0

^aPIXEL energies are for a pair of molecules. The pairs of molecules are defined by the strongest intermolecular interaction;

^belectrostatic (Coulombic) energy;

^cpolarisation energy;

^ddispersion energy;

^erepulsion energy;

^ftotal intermolecular energy: $U_{\text{inter}} = E_C + E_P + E_D + E_R$. The non-additivity of E_P is not included.