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# Can solvated intermediates inform us about nucleation pathways? The case of $\beta$ -*p*ABA $\dagger$

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Classical nucleation theory teaches the idea that molecular clusters form and grow in solution and that depending on prevailing conditions there is a chance for some to grow large enough to overcome the interfacial energy penalty and become mature crystals. However, from such a kinetic analysis, nothing is learnt of the nature of the composition or the molecular packing in such clusters. As a means of addressing this shortcoming consideration has, in the past, been given to the idea that in certain systems crystallography may offer additional, structural, insights. From this approach the notions of ‘nucleation pathway’ or ‘nucleation transition state’ have become useful concepts around which to formulate hypotheses as to how clusters may yield specific molecular packing, resulting for example, in the observation of crystal polymorphs. Here we offer an in-depth crystallographic analysis related to the nucleation of the  $\alpha$  and  $\beta$  polymorphs of *para*-aminobenzoic acid in an attempt to reveal the pathways leading to the two forms. Using a combination of CSD analyses, crystal structure prediction and targeted crystallizations we explore plausible solution pathways to these polymorphs and discuss our results in the light of known kinetic data for the nucleation and growth of this material.

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## Introduction

In recent years significant advances have been made in our ability to measure and interpret the nucleation rates of organic molecules from solutions.<sup>1–3</sup> The use of crystallographic data in the context of kinetic processes goes back to the work of Burgi *et al.*<sup>4</sup> who demonstrated its utility in mapping the reaction co-ordinate during amine-carbonyl nucleophilic addition reactions. Their conclusions were based on the idea ‘that each example can be regarded as a case where the addition reaction has proceeded to a greater or lesser extent but has been frozen in at a certain stage by the intra- or intermolecular constraints imposed by the crystal environment’. A number of attempts have been made to utilise a similar idea in order to explore structural aspects of crystal nucleation. Thus in his 2003<sup>5</sup> paper concerning structures with high *Z'* values, Steed commented that ‘the observed

structure may readily be viewed as a fossil relic of the fastest growing crystal nucleus rather than a thermodynamic minimum structure’. In 2005<sup>6</sup> Banerjee *et al.* took this idea further in reporting the structure of sodium saccharinate where packing disorder was considered to reflect the nature of the nucleus: ‘there is enough in the structure that is unusual for us to believe that it represents a good approximation of what a crystal nucleus looks like’. Similar conclusions have been drawn from the structure of pentafluorophenol polymorphs.<sup>7</sup> In 2014,<sup>8</sup> Steed and Steed reviewed much available data and offered a balanced comment on the idea that metastable crystal forms may be viewed as fossil relics of kinetically determined initial nuclei. They concluded that this is indeed a difficult leap to make. In the case of solvates, however, one might anticipate more success. In 1999 Nangia and Desiraju<sup>9</sup> reflected on the occurrence of solvates in the CSD and announced that in the context of a crystallisation pathway from solution to unsolvated crystal ‘the formation of a solvated crystal may be likened to an interruption of the sequence of events that accompany the ‘normal’ crystallisation’. This idea was taken up by Banerjee *et al.*<sup>10</sup> in a study of an anthracenediol in which they compare the structure of the non-solvated form with that of five solvates and again return to the idea of the solvent as a kinetic mitigator of packing difficulties. Overall, this collection of studies all attempt to offer structural interpretations of processes whose outcomes rely significantly on kinetics. Often even the relative stability of the solid forms

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under consideration is not known or discussed. As far as we are aware the only report that makes any attempt to utilise crystallographic information to track the real-time transient nature of nucleation and growth concerns the crystallisation of trimesic acid from DMSO.<sup>11</sup> This work provided structural characterisation of both the solution and the short-lived metastable solvate to demonstrate that molecules are able to make the jump from solution to the first nucleated solid phase with no change in their solvated coordination. The application of crystallographic data in furthering our understanding of nucleation is thus far from straightforward and fraught with difficulties as expected when using static data to interpret a kinetic process.

Here we have returned to this problem as part of our examination of the nucleation of benzoic acids.<sup>12</sup> We have been particularly concerned with *p*-aminobenzoic acid which crystallises from solution in one of two commonly observed polymorphs.<sup>13</sup> The  $\alpha$  form is based on the carboxylic acid dimer and the  $\beta$  structure utilises COOH $\cdots$ NH<sub>2</sub> interactions, creating tetramer units linked by H-bonded chains.<sup>14</sup> Crystallisation of the former is dominated by growth along the *b*-axis which involves infinite stacks related by translation while in the latter stacked molecules are limited to isolated dimers whose molecules are related by a centre of symmetry. Since the nucleation and growth of  $\alpha$  is apparently ubiquitous from all solvents and at all temperatures<sup>15</sup> some investigations have sought to probe the origin of the motif found in the  $\beta$  structure.<sup>16</sup> It has been noted and confirmed in various studies that  $\beta$  is most reliably crystallised from aqueous solutions at low supersaturations.<sup>16,17</sup> Hence, the question has arisen as to whether water plays some central role in the nucleation pathway to the  $\beta$  structure and indeed, in our earlier paper, we showed computationally how water might stabilise the centrosymmetric *p*ABA dimer held by aromatic stacks.<sup>18</sup> We give further consideration to this question here using a combination of methodologies and invoking the aid of crystallography – CSD analysis, crystal structure prediction (CSP), targeted crystallisation experiments and crystal structure determination. We consider the rarity of the  $\beta$  structure, the potential for various solvent mediated transition states to enable its formation and the possibility that the isolation of new solvated structures will inform our understanding.

## Methods

### Experimental methods

**Chemicals.** HCl ( $\geq 99\%$  purity; 0.1 M, 1 M, 11 M), NaOH ( $\geq 99\%$  purity; 0.1 M and 1 M), acetone ( $\geq 99\%$ ), 1,4-dioxane ( $\geq 99\%$ ), ethanol ( $\geq 99\%$ ) and methanol ( $\geq 99\%$ ), *p*ABA ( $\alpha$  form,  $\geq 99\%$  purity), 4-amino-2-methoxybenzoic acid (2OMe-*p*ABA, 97%), 4-amino-3-hydroxybenzoic acid (2OH-*p*ABA, 97%), 4-amino-2,3,5,6-tetrafluorobenzoic acid (pF-*p*ABA, 99%), 4-amino-2-fluorobenzoic acid (2F-*p*ABA, 99%), 4-amino-2-chlorobenzoic acid (2Cl-*p*ABA, 99%) and 4-amino-3-chlorobenzoic acid (3Cl-*p*ABA, 99%) were purchased from

Sigma-Aldrich. 4-Amino-2-methylbenzoic acid (2Me-*p*ABA, 97%), 4-amino-5-chloro-2-ethoxybenzoic acid (2OEt-5Cl-*p*ABA, 95%) and 4-amino-5-chloro-2-methoxybenzoic acid (2OMe-5Cl-*p*ABA, 95%) were purchased from Fluorochem. Distilled and de-ionised water was prepared in the laboratory using a MERIT-W4000 ion exchanger and PUR1TE Still-Plus distiller. NB: the abbreviations given in parentheses above are used throughout the text to identify the various derivatives.

**Solubility measurements.** The solubilities of pF-*p*ABA, 2F-*p*ABA, 2Cl-*p*ABA and 3Cl-*p*ABA were measured in water at four different temperatures (5, 10, 25 and 40 °C). The solubility of  $\alpha$  *p*ABA as a function of pH (1.5–6.5) was measured at 25 and 10 °C. A gravimetric method was used (see ESI† section 2) and pH measured using a Mettler Toledo AG8603 pH meter ( $\pm 0.01$ ).

**Crystallisation.** 2OMe-*p*ABA, 2Me-*p*ABA, 2OH-*p*ABA, 2OEt-5Cl-*p*ABA, 2OMe-5Cl-*p*ABA and 2Cl-*p*ABA, were crystallised by slow evaporative crystallisation of saturated acetone solutions prepared at room temperature. These solutions were covered with pierced parafilm and left to evaporate until single crystals suitable for structure determination were obtained. 3Cl-*p*ABA was crystallised from methanol using the same method. In attempts to prepare hydrated/solvated forms of *p*ABA and pF-*p*ABA solvent mixtures (90 vol% organic solvent and 10 vol% water) of ethanol/water, methanol/water, acetone/water and dioxane/water were used. 10 ml samples of solutions were allowed to evaporate over 5 day periods at 4 °C and at room temperature. To explore the impact of pH, crystallisation experiments were performed at different pHs (0.77–8.8) and starting compositions based on the measured solubilities. Solutions saturated at different pHs were cooled at a rate of 1.5 °C per minute. The temperature at which crystallisation was observed (9–25 °C) was used to estimate the supersaturation (1.07–1.94). Cooling crystallisations of selected *p*ABA derivatives (pF-*p*ABA, 2F-*p*ABA, 2Cl-*p*ABA and 3Cl-*p*ABA) were performed in water at different supersaturations (1.1, 1.2, 1.35, 1.5, 1.6, 1.8 and 2.0) and at two different temperatures (10 °C and 25 °C). Solutions were prepared by fully dissolving the required amounts of materials in a stirred (300 rpm) jacketed vessel and holding for 1 h at 40 °C, before cooling to the desired crystallisation temperature (10 °C or 25 °C) at a rate of 1 °C h<sup>−1</sup>. Following this, the solutions were maintained at the desired temperature until crystals appeared ( $\sim 24$  h).

Experimental PXRD patterns of isolated solids were compared to those calculated from XRD structures for form identification. Additionally optical microscopy (Zeiss Axioplan) and single crystal XRD, where available, were necessary.

**PXRD.** Powder X-ray diffractograms were recorded, between 5–40° (2 $\theta$ ) in steps of 0.02°, on a Bruker D2-Phaser using a Cu radiation source (1.5418 Å). Samples were ground with a mortar and pestle and mounted on a flat sample holder.



**XRD.** Single crystal XRD was performed on a Rigaku Oxford Diffraction FR-X DW diffractometer equipped with a selectable dual wavelength (MoK $\alpha$   $\lambda$  = 0.71073 Å and CuK $\alpha$   $\lambda$  = 1.5418 Å) rotating anode system Varimax™ microfoc optics. Rigaku Oxford Diffraction CrysAlis-Pro was used for data collection and cell refinement, and the structures were solved and refined by using SHELX and Olex2. Most hydrogen atoms were assigned idealized positions and were included in structure factor calculations. All non-hydrogen atoms were refined anisotropically. In the case of the solvates prevention of solvent loss and subsequent phase transformations was achieved by storing crystals in their mother liquors until required and data collected at 150 K.

### CSD searches

All searches were performed on the CSD best R-factor subset (2020 release) using ConQuest.<sup>19</sup> This subset does not contain structure redeterminations. Conquest searches were carried out with the following filters: crystal structures must have 3D coordinates, no disorder, no errors, they must not be polymeric or contain ions and only organic molecular crystals were searched.

**Hydrogen bonds (HBs) between amino and acid groups (Table 1).** These searches were limited to single component crystal structures whereby the compound contained both an amino (NH<sub>2</sub>) and carboxylic (COOH) acid functional groups. Compounds containing primary amides of the type CONH<sub>2</sub> were not considered since those NH<sub>2</sub> groups behave significantly differently to amino groups. This search returned 102 hits.

**Aromatic stacks (Table 1).** These searches were restricted to single component benzoic acids and identified those containing aromatic stacks in which the centroid – centroid distance is  $\leq 5$  Å (centroid defined as centre of the aromatic ring). This search returned 813 hits.

**pABA derivatives (Table 2).** The CSD was searched for the pABA substructure whereby no specific substituents were placed in positions 2, 3, 5 and 6. Searches were limited to single component crystals. This search returned 9 structures.

**Zwitterionic amino benzoic acids (Table 3).** The CSD was searched for zwitterionic pABA, mABA and oABA substructures with no specified substituents on positions 2, 3, 5 and 6 and 16 structures were retrieved.

**Interactions within solvates (Table 4).** The CSD was searched for hydrates of compounds (two components only,

the hydrate and the main component) with no charges and containing the following substructures: i) benzoic acid (179 hits), ii) aniline (120 hits) and compounds containing both an amine and an acid group (amine-X-acid, 60 hits). There was no significant overlap between these searches.

**Choice of crystallisation solvents for the isolation of hydrates (Table 8).** To aid the process of solvent selection for crystallisation we utilised experimental data recorded in the CSD cif files. To do this two CSD searches (CSD2015) were performed. The first identified compounds containing a carboxylic acid group crystallising in anhydrous forms (7225 structures) and the second compounds containing a carboxylic acid group crystallising as hydrates (1115 structures). Structures containing information on solvent of crystallisation were kept (1864 anhydrous and 206 hydrates). The data was analysed per solvent of crystallisation and a probability of a hydrate being obtained was derived as the ratio between the crystallisations resulting in a hydrate (number of hydrates obtained) over the total number of crystallisations carried out in that solvent (resulting in either anhydrous or hydrate forms).

### CSD materials calculations

The following calculations were all performed with the CSD Materials Mercury (2020).<sup>20</sup>

**Hydrogen bond propensities (HBPs).** The HBP tool<sup>21,22</sup> allows the ranking of a structure in terms of the likelihood of its intermolecular interactions. It applies a statistical analysis to structures in the CSD to determine quantitatively the likelihood of hydrogen bond formation between individual functional groups of the target molecule. Included in the calculation is the coordination score: for each functional group, the coordination likelihood captures whether the functional group will participate in any number of hydrogen bond interactions. A chart, plotting all possible combinations of hydrogen bond donors with acceptors for the target molecule, ranked in terms of propensity and coordination score highlights where the observed solid form resides in a landscape of possible hydrogen bond networks.<sup>22</sup> This is used in our analysis of hydrogen bonding motifs amongst molecules with amino (NH<sub>2</sub>) and carboxylic (COOH) acid functional groups.

**Aromatic analyser.** The aromatics analyser tool<sup>20</sup> analyses the contribution of aromatic interactions to the stability of a crystal structure. The feature uses an artificial neural network

**Table 1** Statistics on the popularity of the primary and secondary HB motifs and aromatic stack geometries found in the  $\alpha$  and  $\beta$  pABA forms

| Form                    | Primary HBs |                        | Secondary HBs          | Aromatic stacks |              |
|-------------------------|-------------|------------------------|------------------------|-----------------|--------------|
|                         | $\alpha$    | $\beta$                | $\alpha$ and $\beta$   | $\alpha$        | $\beta$      |
| Interaction             | COOH...COOH | COOH...NH <sub>2</sub> | NH <sub>2</sub> ...O=C | Translated      | Inverted     |
| CSD stats (102 hits)    | 46%         | 3%                     | 36%                    | 38%             | 62%          |
| HB propensity           | 0.30        | 0.04                   | 0.53                   | —               | —            |
| Aromatic analyser score | —           | —                      | —                      | 9.7 (strong)    | 9.2 (strong) |
| CSP stats               | 98%         | 2%                     | —                      | 52%             | 5%           |



**Table 2** Summary of crystal structures and interactions found in *p*ABA, *p*ABA derivatives in the CSD and newly determined derivatives in this study

| Compound/form                                       | CSD refcode | Z' | SG           | Primary HB (donor-acceptor) | Secondary HB (donor-acceptor) | Stacking           |
|-----------------------------------------------------|-------------|----|--------------|-----------------------------|-------------------------------|--------------------|
| <i>p</i> ABA forms                                  |             |    |              |                             |                               |                    |
| $\alpha$ - <i>p</i> ABA                             | AMBNAC07    | 2  | $P2_1/n$     | Acid-acid                   | Amino-O=C                     | t [ $\infty$ ]     |
| $\gamma$ - <i>p</i> ABA                             | AMBNAC09    | 2  | $Pna2_1$     | Acid-acid                   | Amino-O=C                     | t [ $\infty$ ]     |
| $\beta$ - <i>p</i> ABA                              | AMBNAC12    | 1  | $P2_1/n$     | Acid-amino                  | Amino-O=C                     | i                  |
| $\delta$ - <i>p</i> ABA                             | AMBNAC16    | 1  | $Pn$         | Acid-amino                  | Amino-O=C                     | t [ $\infty$ ]     |
| <i>p</i> ABA derivatives with $\alpha$ similarities |             |    |              |                             |                               |                    |
| 3-Br                                                | HOLTAE      | 2  | $Pna2_1$     | Acid-acid                   | Amino-O=C                     | t [ $\infty$ ]     |
| 3-iProp                                             | JISGAW      | 1  | $P2_1/c$     | Acid-acid                   | Amino-O=C                     | t [ $\infty$ ]     |
| 3-LC <sup>a</sup>                                   | DUMYIV      | 1  | $P2_1/n$     | Acid-acid                   | Amino-O=C                     | t [ $\infty$ ]     |
| 3,5-diF                                             | YOZFIE      | 1  | $Pmna$       | Acid-acid                   | Amino-O=C                     | t [ $\infty$ ]     |
| 3,5-diBr                                            | BRABZA01    | 1  | $Pman$       | Acid-acid                   | Amino-O=C                     | t [ $\infty$ ]     |
| 3,5-diI                                             | YOZFUQ01    | 1  | $Pmna$       | Acid-acid                   | Amino-O=C                     | t [ $\infty$ ]     |
| 2-F                                                 | This study  | 4  | $C2/c$       | Acid-acid                   | Amino-amino                   | t [ $\infty$ ]     |
| 2-Cl                                                | AYOSOX      | 2  | $P2_1$       | Acid-acid                   | Amino-amino                   | t [ $\infty$ ]     |
| 2-OH                                                | AMSALA02    | 1  | $P2_1/n$     | Acid-acid                   | Other                         | t [ $\infty$ ]     |
| 3-Cl                                                | This study  | 1  | $P2_1/c$     | Acid-acid                   | Other                         | t [ $\infty$ ]     |
| 3-NO <sub>2</sub>                                   | PUQFUD      | 1  | $P2_1/n$     | Acid-acid                   | Other                         | i [ $\infty$ ]     |
| 2-Me                                                | This study  | 2  | $P2_12_12_1$ | Acid-acid                   | Amino-amino                   | Other              |
| 3-Me                                                | This study  | 2  | $P2_1/c$     | Acid-acid                   | Amino-O=C                     | t                  |
| pF (2,3,5,6-tetraF)                                 | This study  | 1  | $P2_1/c$     | Acid-acid                   | Amino-O=C                     | t [ $\infty$ ]     |
| <i>p</i> ABA derivatives with $\beta$ similarities  |             |    |              |                             |                               |                    |
| 3-OH                                                | This study  | 1  | $Pbca$       | Acid-amino                  | Amino-O=C                     | Other [ $\infty$ ] |
| Other <i>p</i> ABA derivatives                      |             |    |              |                             |                               |                    |
| 2-OMe                                               | This study  | 1  | $P2_1/c$     | Other intra                 | Amino-O=C                     | Other [ $\infty$ ] |
| 2-OMe-5-Cl                                          | This study  | 1  | $P2_1/c$     | Other intra                 | Amino-O=C                     | Other [ $\infty$ ] |
| 2-OEt-5-Cl                                          | This study  | 1  | $P\bar{1}$   | Other intra                 | Amino-O=C                     | g [ $\infty$ ]     |

<sup>a</sup> LC = complex long chain; t = translation; i = inversion; g = glide plane. Continuous interactions are given an infinity sign in brackets. See ESI† section 1 for CSD deposition numbers of the new structures.

**Table 3** Summary of interactions found in *p*ABA, *m*ABA and *o*ABA derivatives crystallising as zwitterionic forms

|           | Substructure <sup>a</sup> | CSD Refcode | Components <sup>a</sup>                                                | Primary HB                                       | HB tetramer    | Aromatic stacking           |
|-----------|---------------------------|-------------|------------------------------------------------------------------------|--------------------------------------------------|----------------|-----------------------------|
| Anhydrous | <i>o</i> ABA-z            | AMBACO07    | <i>o</i> ABA-z <i>o</i> ABA-uc                                         | NH <sub>3</sub> <sup>+</sup> ...COO <sup>-</sup> | No             | Other                       |
|           | <i>m</i> ABA-z            | AMBNZA02    | <i>m</i> ABA-z                                                         | NH <sub>3</sub> <sup>+</sup> ...COO <sup>-</sup> | Yes            | t [ $\infty$ ]              |
|           | <i>m</i> ABA-z            | SAQJAC01    | 6-OH- <i>m</i> ABA-z                                                   | NH <sub>3</sub> <sup>+</sup> ...COO <sup>-</sup> | Yes            | t [ $\infty$ ]              |
|           | <i>m</i> ABA-z            | DOBPAO      | 2-NH <sub>2</sub> - <i>m</i> ABA-z 2-NH <sub>2</sub> - <i>m</i> ABA-uc | NH <sub>3</sub> <sup>+</sup> ...COO <sup>-</sup> | No             | i                           |
|           | <i>m</i> ABA-z            | VODWIU      | 4-NH <sub>2</sub> - <i>m</i> ABA-z                                     | NH <sub>3</sub> <sup>+</sup> ...COO <sup>-</sup> | Yes            | t [ $\infty$ ]              |
|           | <i>m</i> ABA-z            | EQICOY      | 2-OH- <i>m</i> ABA-z                                                   | NH <sub>3</sub> <sup>+</sup> ...COO <sup>-</sup> | Yes            | t [ $\infty$ ]              |
|           | <i>p</i> ABA-z            | PEJYEJ      | 3-X <sup>b</sup> - <i>p</i> ABA-z                                      | NH <sub>3</sub> <sup>+</sup> ...COO <sup>-</sup> | Yes, variation | Other                       |
| Hydrates  | <i>m</i> ABA-z            | POVGUC02    | 5-COOH- <i>m</i> ABA-z water                                           | NH <sub>3</sub> <sup>+</sup> ...COO <sup>-</sup> | No             | 2 <sub>1</sub> [ $\infty$ ] |
|           | <i>m</i> ABA-z            | SOYPOM      | 4-OH- <i>m</i> ABA-z water                                             | NH <sub>3</sub> <sup>+</sup> ...COO <sup>-</sup> | No             | i                           |

<sup>a</sup> uc = uncharged; z = zwitterionic. <sup>b</sup> X = complex substituent.

**Table 4** Summary of preferred hydrogen bonding and stacking interactions in hydrates of different searches containing benzoic acid/aniline/amide-X-acid and water in the CSD

| Conquest search query | N   | Hydrogen bonds                          |                                                                 |                                                     | Aromatic stacks |        |
|-----------------------|-----|-----------------------------------------|-----------------------------------------------------------------|-----------------------------------------------------|-----------------|--------|
|                       |     | (COOH...COOH) s, 0.5 s, us <sup>a</sup> | (NH <sub>2</sub> ...NH <sub>2</sub> ) s, 0.5 s, us <sup>a</sup> | (COOH...NH <sub>2</sub> ) s, 0.5 s, us <sup>a</sup> | s-S[t]          | s-S[i] |
| Benzoic acid : water  | 179 | 14%, 9%, 18%                            | —                                                               | —                                                   | 33%             | 35%    |
| Aniline : water       | 120 | —                                       | 8%, 0%, 2%                                                      | —                                                   | 28%             | 32%    |
| Amine-X-acid : water  | 60  | 3%, 0%, 7%                              | 0%, 0%, 0%                                                      | 0%, 0%, 13%                                         | —               | —      |

<sup>a</sup> s = solvated dimer with two water molecules; 0.5 s = half solvated dimer with one water molecule; us = unsolvated dimer. Thus, for the acid...acid dimer, the fully solvated motif contains 2 molecules of water and 2 molecules of acid, the half solvated motif contains 1 molecule of water and 2 molecules of acid and the unsolvated motif is the typical unsolvated and R<sub>2</sub><sup>2</sup>(8) dimer.





**Table 5** Summary of motifs found in hypothetical structures of 1:1 and 1:2 water : *p*ABA. HB motifs are always given as donor...acceptor

|                                                                        |                              | CSP structures          |                         |
|------------------------------------------------------------------------|------------------------------|-------------------------|-------------------------|
|                                                                        |                              | 1:1 <i>p</i> ABA: water | 1:2 <i>p</i> ABA: water |
| Lowest Elatt (kJ mol <sup>-1</sup> )                                   |                              | -177.8                  | -233.8                  |
| <i>N</i> structures within 20 kJ mol <sup>-1</sup> from global minimum |                              | 299                     | 205                     |
| Alpha motifs                                                           | COOH...COOH dimer            | 68%                     | 31%                     |
|                                                                        | Alpha stack                  | 31%                     | 16%                     |
| Beta motifs                                                            | COOH...NH <sub>2</sub> dimer | 9%                      | 1%                      |
|                                                                        | Inverted stack               | 2%                      | 3%                      |
| Alpha and beta                                                         | NH <sub>2</sub> ...COOH (O=) | 22%                     | 1%                      |
|                                                                        | COOH...w                     | 30%                     | 71%                     |
| Other hydrated interactions                                            | NH <sub>2</sub> ...w         | 92%                     | 93%                     |
|                                                                        | w...NH <sub>2</sub>          | 74%                     | 89%                     |
|                                                                        | w...COOH(O=)                 | 63%                     | 96%                     |

**Table 6** Calculated free energy for hydrate formation for 1:1 and 1:2 *p*ABA : water

|                          | $\Delta G$ hydrate formation (kJ mol <sup>-1</sup> of hydrate) |
|--------------------------|----------------------------------------------------------------|
| 1:1 <i>p</i> ABA : water | 7.3                                                            |
| 1:2 <i>p</i> ABA : water | 15.1                                                           |

**Table 7** Analogues of acetic, succinic and terephthalic acids and the CSD refcodes for their anhydrous and hydrated forms

| Analogues                   | R                                        | Anhydrous | Hydrates       | Hydrate stoichiometry |
|-----------------------------|------------------------------------------|-----------|----------------|-----------------------|
| Acetic acid analogues       | CH <sub>3</sub>                          | ACETAC    | —              | —                     |
|                             | CH <sub>2</sub> F                        | FACETC    | —              | —                     |
|                             | CH <sub>2</sub> Cl                       | CLACET    | —              | —                     |
|                             | CH <sub>2</sub> Br                       | BRMACA    | —              | —                     |
|                             | CHCl <sub>2</sub>                        | YIQGAH    | —              | —                     |
|                             | CH <sub>2</sub> Br                       | BARBAX    | —              | —                     |
|                             | CFClBr                                   | OHIJAR    | —              | —                     |
|                             | CBr <sub>3</sub>                         | WADFIR    | —              | —                     |
|                             | CCl <sub>3</sub>                         | TCACAD    | —              | —                     |
|                             | CFCl <sub>2</sub>                        | NAGVUM    | RABBUN         | 1:0.5                 |
|                             | CF <sub>2</sub> Cl                       | NAGWAT    | RABCAU, RABCEY | 1:1, 1:4              |
| Succinic acid analogues     | CF <sub>3</sub>                          | TFACET    | BULMAW10       | 1:1                   |
|                             | CH <sub>2</sub> -CH <sub>2</sub>         | SUCACB    | —              | —                     |
|                             | CHCl-CH <sub>2</sub>                     | CLSUC     | —              | —                     |
|                             | CHBr-CH <sub>2</sub>                     | WANEE     | —              | —                     |
|                             | CHBr-CHBr                                | SAZSES    | —              | —                     |
|                             | (trans) CHBr-CHBr                        | WOCHIF    | —              | —                     |
|                             | (syn) CHF-CHF                            | VEVSIZ    | —              | —                     |
|                             | (syn) CHF-CHF                            | —         | VEVSUL         | 1:2                   |
|                             | (trans) CF <sub>2</sub> -CF <sub>2</sub> | —         | ZESZUS01       | 1:1                   |
|                             | H, H, H, H                               | TEPHTH    | —              | —                     |
| Terephthalic acid analogues | Cl, Cl, Cl, Cl                           | CIPZIL    | —              | —                     |
|                             | H, Br, Br, H                             | —         | POFROS         | 1:2                   |
|                             | F, F, F, F                               | BITCEM    | YABHOA         | 1:2                   |

to provide a quantitative assessment of each aromatic interaction in comparison to the best geometry that could be achieved for a phenyl...phenyl contact. The model is based on a geometric description of aromatic interactions involving the position of the two phenyl rings relative to each other. The outcome of this model is a score (from 0 to 10) capturing the range of weak (0–3), moderate (3–7) or strong (7–10) interactions. This aided our analysis of aromatic contacts amongst molecules with amino (NH<sub>2</sub>) and carboxylic (COOH) acid functional groups.

**Solvate prediction tool.** The solvate prediction tool<sup>23</sup> estimates the probability of solvate formation, based purely on molecular structure. The model is based on 2D molecular descriptors and uses a balanced data set generated using a random undersampling method. These predictions should be considered as a statistical tendency towards, or away from, formation of a solvate, not a strong confirmation of one outcome or the other.

## Molecular simulations

**Crystal structure prediction (CSP).** CSP calculations for all systems ((1) *p*ABA ((a) *Z'* = 1, (b) *Z'* = 2), (2) water, (3) *p*ABA : water 1:1 ratio, (4) *p*ABA : water 1:2 ratio) were carried out using the code CrystalPredictor<sup>24</sup> (version 2.4.3). Flexibility was determined using gas phase finite difference perturbations around degrees of freedom indicated as potentially flexible by second derivatives at the gas phase minimum; this meant the carboxylic acid group torsion. The level of theory used was B3LYP, with the Aug-CC-pVTZ basis set, in Gaussian 09. A uniform local approximate model (LAM) grid was set up for *p*ABA, with LAMs evaluated at 0.0 and 180.0 degrees; a pass of the adaptive LAM algorithm<sup>25</sup> indicated that this was sufficient to accurately describe the flexibility within the molecules. The potential parameters for C, H-C (hydrogen attached to carbon), N, O, and H-n (hydrogen attached to a polar atom) from the work of Williams and co-workers were used to describe the exchange-repulsion and dispersion interactions.<sup>26</sup> The structure generation stage sampled the 59 most common space groups. 500 k and 1 million structural minimisations were run for the *Z'* = 1 (1a, 2, 3, 4 and 5) and 2 (1b) searches respectively, using the smoothed intramolecular potential algorithm.<sup>27–29</sup> After the CrystalPredictor calculations were complete, a final clustering of generated structures was carried out with the COMPACT algorithm. In order to refine the calculated lattice energies, the generated structures were minimised with an improved energy model for electrostatics consisting of atomic multipoles, with extended flexibility (amine hydrogen angles, and all angles within the carboxylic acid group), together with the same FIT potential, using CrystalOptimizer.<sup>30</sup> The same level of theory (B3LYP/Aug-CC-pVTZ) was employed (see also ESI† section 3.1).

**Analyses of motifs in the CSP structures.** Motifs were analysed using CSD Mercury with the motif analysis and the 3D feature search tools.



**Table 8** CSD analysis of crystallisation solvents and hydrate formation

| Crystallisation solvent  | Crystallisation outcome |                |       | Hydrate crystallisation probability |
|--------------------------|-------------------------|----------------|-------|-------------------------------------|
|                          | Anhydrous acids         | Hydrated acids | Total | Hydrates/total (%)                  |
| Methanol                 | 211                     | 24             | 235   | 10                                  |
| Ethanol                  | 230                     | 18             | 248   | 7                                   |
| Water                    | 108                     | 14             | 122   | 12                                  |
| Acetone                  | 120                     | 2              | 122   | 2                                   |
| Ethyl acetate            | 105                     | 9              | 114   | 2                                   |
| Ethanol/water            | 77                      | 35             | 112   | 31                                  |
| Hexane/ethyl acetate     | 63                      | 2              | 65    | 3                                   |
| Methanol/water           | 43                      | 12             | 55    | 22                                  |
| Chloroform               | 48                      | 2              | 50    | 4                                   |
| Diethyl ether            | 40                      | 2              | 42    | 5                                   |
| Chloroform/methanol      | 19                      | 2              | 21    | 10                                  |
| Toluene                  | 16                      | 3              | 19    | 16                                  |
| Methanol/dichloromethane | 14                      | 3              | 17    | 18                                  |
| Acetone/water            | 11                      | 6              | 17    | 35                                  |

**Improved lattice energy calculations (TPSS-D3) for a selected predicted structures subset.** As a final step, the lattice energies of the  $\alpha$  and  $\beta$  polymorphs of *p*ABA together with ice (hexagonal and cubic) and the five lowest energy hydrate structures from investigations 3 and 4, were recomputed with periodic density functional theory with van der Waals corrections. For this, the TPSS functional was used, with Grimme's D3 vdW corrections, as implemented in the VASP code (version 5.4). An energy cut off of 1000 eV was used for the plane waves. The Brillouin zone was sampled using a Gamma centred Monkhorst–Pack approximation at  $k$ -point grids separated by approximately  $0.025 \times 2/\pi \text{ \AA}^{-1}$ . Crystal structures were relaxed with this model allowing the unit cell volume as well as the atomic positions to optimise. Structural relaxations were halted when the calculated force on every atom was less than  $0.01 \text{ eV \AA}^{-1}$  (see also ESI† sections 3.1 and 4.8).

## Results

### On the rarity of the $\beta$ -*p*ABA structural motifs

The major objective of this section is the use of the CSD searches to explore the rarity or otherwise of the  $\beta$  *p*ABA structural motifs. Fig. 1 provides images of the two key motifs that constitute the  $\beta$  structure. The first motif (Fig. 1a) is a hydrogen bonded  $R_4^4(12)$  tetramer constituted by two  $\text{COOH}\cdots\text{NH}_2$  primary hydrogen bonds and two  $\text{NH}_2\cdots\text{O}=\text{C}$  secondary hydrogen bonds (primary and secondary are used

to underline the fact that  $\text{NH}_2\cdots\text{O}=\text{C}$  is expected to be weaker and less directing than the  $\text{COOH}\cdots\text{NH}_2$ ) the second motif (Fig. 1b) is the aromatic stacking interaction in which molecules are related by inversion symmetry. As mentioned earlier these differ significantly from the  $R_2^2(8)$  HB dimer and translated aromatic stacks found in the  $\alpha$  polymorph.

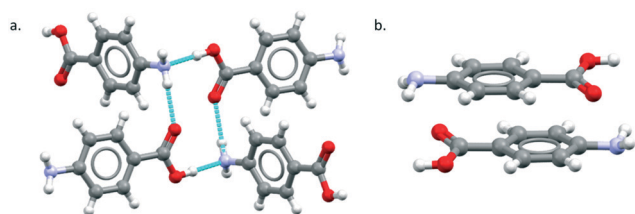
Table 1 provides a summary of the search results and related data for crystal structures of compounds containing both an amino ( $\text{NH}_2$ ) and carboxylic ( $\text{COOH}$ ) acid functional groups, indicating the overall occurrence of the relevant motifs as found in the  $\alpha$  and  $\beta$  structures.

In terms of the HB, in searches of a total of 102 structures the  $R_2^2(8)$  carboxylic acid dimer motif is the most common (46%) followed by the secondary  $\text{NH}_2\cdots\text{O}=\text{C}$  interactions (36%) and the  $\text{COOH}\cdots\text{NH}_2$  primary hydrogen bond (3%). In fact, only three crystal structures were observed to have the  $\text{COOH}\cdots\text{NH}_2$  hydrogen bond, two of which were the  $\beta$  and the  $\delta$  *p*ABA polymorphs. These overall statistics marry well with the HBP analysis which predicts the secondary  $\text{NH}_2\cdots\text{O}=\text{C}$  interaction as having the highest propensity followed by the  $R_2^2(8)$  carboxylic acid dimers. The propensity for the  $\text{COOH}\cdots\text{NH}_2$  interaction to form is very low (0.04) confirming the rarity of this motif. Turning to the aromatic interactions, the CSD statistics and aromatic analyser shows that stacks related by both translation and inversion are common and that the geometries of such stacks in  $\alpha$  and  $\beta$  *p*ABA lead to strong aromatic interactions.

Finally, an analysis of HB and aromatic stacking motifs in the CSP landscape for *p*ABA generated in previous work (structures within  $10 \text{ kJ mol}^{-1}$  of the global minimum) reveals that, both the  $\text{COOH}\cdots\text{NH}_2$  and the inverted aromatic stacks as found in the  $\beta$ -*p*ABA forms are rare.<sup>13</sup>

### *p*-Aminobenzoic acid derivatives

Beyond the overall statistics (Table 1) for compounds with amino and a carboxylic acid groups we also explored the specific motifs found in crystal structures of *p*-aminobenzoic acid derivatives. The CSD search returned 13 hits: the four



**Fig. 1**  $\beta$  *p*ABA motifs: (a)  $R_4^4(12)$  and (b) the centrosymmetric stacked dimer.



known polymorphs of *p*ABA plus nine derivatives. A search of the same substructure in Sigma Aldrich returned a number of *p*ABA derivatives which were not available in the CSD. Nine of those (chosen based on affordability) were purchased, single crystal structures determined and their solubilities measured in water (see Methods and ESI† section 2). Overall, this combined search and experimentation yielded 18 structures of *p*ABA derivatives. Table 2 provides a summary of the crystallographic data including  $Z'$  values, space groups and the major motifs. Here the relative rarity of the  $\beta$  motifs is again evident. Of the 18 derivatives, 12 show carboxylic acid  $R_2^2(8)$  dimers together with infinite stacking of the aromatic rings *via* translation. This reflects our previous conclusions for *p*ABA and other *para* substitute aromatic acids, of the importance of infinite (one dimensional) aromatic stacking in controlling the kinetics of nucleation and growth since the zero-dimensional carboxylic acid dimers cannot, alone, enable extension of the crystal structure.<sup>12</sup> Two other derivatives (2-Me and 3-Me *p*ABA) are also based on carboxylic acid  $R_2^2(8)$  dimers but their continuous interactions which drive growth comprise a more complex mixture of stacks and  $\text{CH}\cdots\text{aromatic}$  contacts. A further three derivatives (2-OMe, 2-OMe-5-Cl, 2-OEt-5-Cl) have either a methoxide or an ethoxide substituent on carbon 2 which results in the carboxylic acid geometry being *anti* rather than *syn* so as to form an intramolecular  $\text{COOH}\cdots\text{OMe/OEt}$  hydrogen bond. The dominant interactions in these structures are the aromatic stacks, which are all strong and continuous, adopting different symmetries. Only one of the *p*ABA derivatives (4-amino-3-hydroxyl-benzoic acid, 3-OH-*p*ABA) was found to have a  $\text{COOH}\cdots\text{NH}_2$  interaction similar to that found in the  $\beta$  form. This acid-amine interaction, however, does not assemble into the  $\beta$  HB tetramer (Fig. 1a). Instead, through involvement of the hydroxyl group, the structure displays a different kind of infinite hydrogen bonding arrangement as seen in Fig. 2.

Thus, as with compounds containing both an amino ( $\text{NH}_2$ ) and carboxylic ( $\text{COOH}$ ) acid functional groups, this family of substituted *p*ABA structures confirm that the  $\beta$  *p*ABA motifs are essentially unique. Because earlier work

reported that  $\beta$  could only be reproducibly obtained from aqueous solution at very specific, low, supersaturations we also crystallised *pF-p*ABA, 2F-*p*ABA, 2Cl-*p*ABA and 3Cl-*p*ABA at a range of supersaturations (see Methods) and temperatures (10 °C and 25 °C) to check the outcome. In all cases, the forms obtained corresponded to those crystallised by slow evaporation from acetone/methanol and thus were analogous to the  $\alpha$ -*p*ABA structure. There was thus no evidence at either temperature or at low supersaturations, of a  $\beta$ -like structure.

In summary, the acid-amine interaction, hydrogen bonded tetramer and inverted stacking motifs found in the  $\beta$ -*p*ABA structure are rare, both within the group of substituted *p*-aminobenzoic acids and within the CSD as a whole. This conclusion leads us, in the next section, to speculate on possible specific assembly pathways that may underpin the appearance of the  $\beta$  structure, particularly from aqueous solutions.

### Potential molecular self-assembly pathways leading to the various *p*ABA motifs

In an attempt to understand the difficulty of crystallisation of  $\beta$  *p*ABA from solution, we hypothesise and discuss here a number of possible self-assembly pathways (Fig. 3).

First, in solution, the neutral *p*ABA molecule is in equilibrium with its zwitterionic form (Fig. 3 central box) which may assemble *via* coulombic ( $\text{COO}^-\cdots\text{NH}_3^+$ ) interactions followed by desolvation leading to the  $\beta$  form. Clearly, like charges would preclude such a route to the HB acid dimer. Such assembly may occur *via* direct head to tail interactions yielding  $s\text{-(COO}^-\cdots\text{NH}_3^+)$  species two of which would make the HB tetramer and/or through aromatic stacking forming  $s\text{-zwit-S[i]}$  species, where *s* refers to solvation and *S* to stacks. Once these species form, conversion to the  $\beta$  *p*ABA motifs only requires direct proton transfer. This is referred to as the zwitterionic pathway.

Second, we explore the possibility that some solvent mediated molecular assemblies may be relatively stable as intermediate states. Since both  $\alpha$  and  $\beta$  forms can be obtained from water (albeit at different supersaturations) the possibility that water plays a key role in creating a transient, hydrated, intermediate seems worth exploring. This possibility has been discussed previously<sup>18</sup> and Fig. 3 shows two pathways from hydrated intermediates to either the  $\beta$  or the  $\alpha$  polymorph. These two different routes involve the formation of stable solvated aromatic stacks (related by translation for  $\alpha$  and inversion for  $\beta$ ) and the formation of solvated carboxylic acid dimers for the  $\alpha$  form and solvated acid-amine dimers for the  $\beta$  form. Such an assembly would then transform to either the  $\alpha$  or the  $\beta$  structure by simple dehydration or concerted dehydration and insertion of amine groups or acid groups.

To explore the reality of these two pathways, we used a combination of CSD searches for appropriate zwitterionic and hydrated motifs, crystallisation conditions aimed at exploiting both hypothetical zwitterionic and hydrate routes

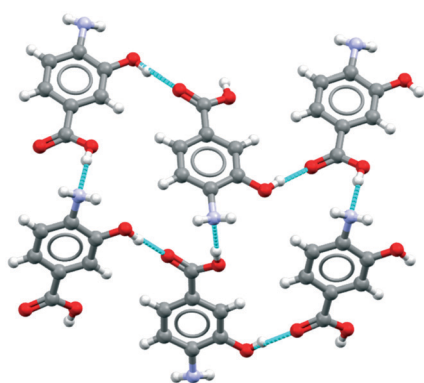


Fig. 2 Hydrogen bonding observed in the crystal structure of 4-amino-3-hydroxyl-benzoic acid (3-OH-*p*ABA).



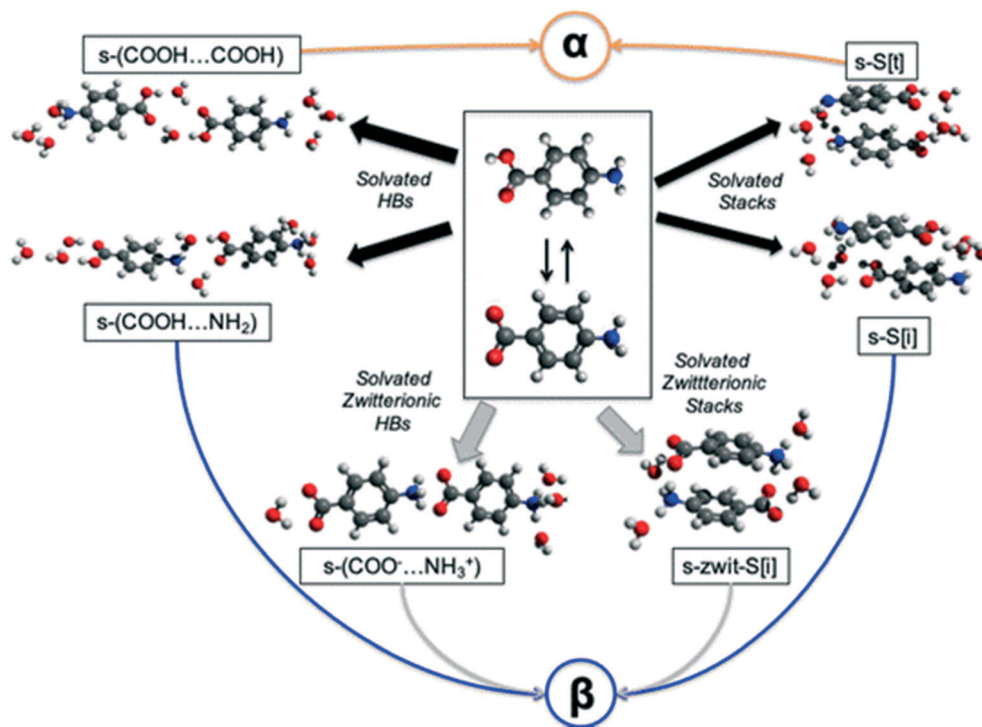


Fig. 3 Plausible pathways for the solution assembly of the  $\alpha$  and  $\beta$  motifs in *pABA*. Blue, orange, black and grey indicate  $\beta$ ,  $\alpha$ , hydrated and zwitterionic pathways respectively.

and CSP calculations searching for potential hydrated structures.

### The zwitterionic pathway

**CSD searches.** In seeking to confirm or eliminate the role of zwitterionic species in the formation of the  $\beta$  polymorph (outlined above and in Fig. 3, grey pathway) we first searched the CSD for zwitterion based structures containing either the *para*, *meta* or *ortho* aminobenzoic acid substructure. This resulted in 16 hits, 9 of which, (selected based on simplicity, *i.e.* smaller substituents) for anhydrous single component crystals and hydrates, are recorded in Table 3 together with a summary of their essential motifs.

These data show that the  $\text{NH}_3^+\cdots\text{COO}^-$  dimer, a potential primary building block for the  $\beta$  tetramer, is present in all of the zwitterionic structures. However, analysis of the aromatic

stacking also shows that the zwitterionic forms, like the uncharged species, prefer to form infinite stacks *via* translation (as found in  $\alpha$ -*pABA*) rather than the centrosymmetric stacks typical of the  $\beta$  form. Most interestingly perhaps, the tetramer is not present in hydrates because of direct hydration of this zwitterionic motif.

Fig. 4 illustrates examples of these effects as seen in the *mABA* hydrate, showing the HB and stacking motifs. Thus in Fig. 4a the hypothesized zwitterionic tetramer of Fig. 3 is indeed evident but in combination with the translated stacks of Fig. 4b. We note from Table 3 that there are plenty of examples of zwitterionic *oABA* and *mABA* structures. In fact, these compounds are known to be able to crystallise in both the zwitterionic and uncharged forms (being rare examples of zwitterionic polymorphs). The only *pABA* derivative being zwitterionic, however, has an extra amino group in the meta position, thus, it can also be described as a *mABA* derivative.

Notwithstanding the small number of observations, we may conclude that this analysis fails to give any clear support to the zwitterionic pathway or to reveal why water may play a key role in the appearance of  $\beta$ .

***pABA* speciation.** An experimental avenue for testing the zwitterionic route requires a knowledge of the speciation of *pABA* as a function of pH. This is revealed through its acid ionisation constants  $p\text{ABA}^+$  (deprotonation of  $\text{NH}_3^+$ ,  $K_1$ ) and  $p\text{ABA}$  (deprotonation of the acid,  $K_2$ ) species together with the microscopic equilibrium constant,  $K_z$  for the equilibrium, eqn (1), between neutral and zwitterionic species. Given these data, the possibility of using pH as a variable to change the

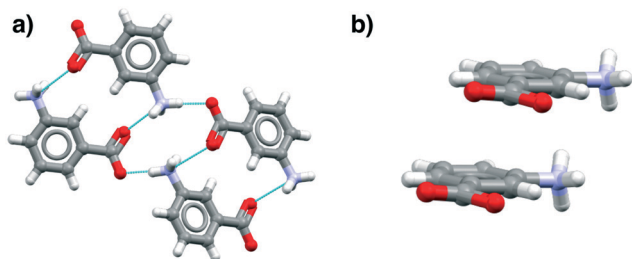
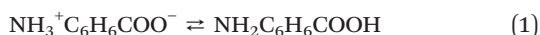


Fig. 4 The zwitterionic tetramer (a) and translated stack (b) in *mABA* (CSD refcode AMBZA01).





zwitterion concentration during crystallisation may be assessed. Robinson and Biggs<sup>31</sup> and van de Graaf *et al.*<sup>32</sup> summarised the reported values of  $pK_1$  and  $pK_2$  in water to be between 2.4–2.6 and 4.7–4.9. From their estimated values of  $K_z$ , the proportion of zwitterions lies between 10 and 17% of all neutral molecules. Using  $pK$  values of 2.5 and 4.87 the speciation diagram in Fig. 5 has been constructed together with the calculated pH dependent ideal solubility.<sup>33</sup> Actually our solubility measurements at 25 °C gave values (see ESI† section 2) of 0.035  $\text{ML}^{-1}$  at pH 3.8 (the isoelectric point, pI), rising to 0.071 at pH = 2.5 and 0.054  $\text{ML}^{-1}$  at pH = 4.85 indicating good agreement (*cf.* Fig. 5) with the solubility prediction.



If we assume, for the moment, that 10% of these neutral molecules are in the zwitterionic state (this is determined by  $K_z$  and independent of pH) then, using Fig. 5, we may consider whether it is possible to use pH to significantly increase the zwitterion concentration and hence direct the outcome, *via* the potential zwitterionic route, to favour the  $\beta$  form. For example, at the pI, and given the solubility-pH values of Fig. 5, the concentration of zwitterions in a saturated solution is  $\sim 0.0032 \text{ ML}^{-1}$ . At the  $pK$ s (2.5 and 4.87) this value changes very little to 0.0036 and 0.0027  $\text{ML}^{-1}$  respectively. Even at more extreme values of pH, 1.5 and 5.5 these values only fall as low as 0.002 and as high as 0.004  $\text{ML}^{-1}$ . Thus, the combination of rising solubility and falling proportion of neutral molecules, as the pH moves away from pI, combine to prevent us from performing crystallisation experiments at significantly different zwitterion concentrations. Hence, we conclude that such an experimental strategy will be of no use in testing this mechanism. Indeed, the results of cooling crystallisations at fixed pH (10 and 25 °C) confirm that within the range 1.2 to 6.6, pH has no impact on the polymorphic outcome compared to the known outcomes at pH 3.8 (pI)<sup>17</sup> with low supersaturations always giving the  $\beta$  form and high

supersaturations the  $\alpha$  polymorph (Nb. outside this pH range for  $7 < \text{pH} < 1$  the hydrochloride and sodium salts were isolated).

Despite this situation in which no direct test of the mechanism is possible we do note two related features. Firstly, van de Graaf *et al.*<sup>32</sup> not only reported  $K_z$  for *pABA* in pure aqueous solution but also in ethanol/water mixtures (50 and 75% ethanol). The percentage of zwitterions was estimated as 17% in pure water, falling to 0.27 and 0.015% with increasing alcohol content. Such decreasing percentages of zwitterion with increasing alcohol content does appear to be consistent with a zwitterion route since the  $\beta$  form is never crystallised from ethanol, irrespective of supersaturation.<sup>17</sup> Further to this we note that, in aqueous solutions of *mABA* and *oABA* the proportion of zwitterions is significantly higher than in *pABA* with 73 and 80% respectively of neutral molecules being in the zwitterionic state.<sup>34,35</sup> As mentioned above, both of these compounds crystallise in zwitterionic forms (Table 3, AMBNZA02, AMBACO07).

Our overall conclusions with regards the role of zwitterions are thus mixed. On the one hand, consideration of the CSD indicates little evidence for the zwitterionic tetramer in the solid forms of aminobenzoic acids and that when it is present it is not combined with inversion stacking. At the same time, the combination of speciation and solubility conspire to make a direct experimental test of this mechanism impossible. However, the appearance of zwitterionic structures and the difficulty in crystallising the  $\beta$  form of *pABA* do appear to show tentative links to the proportion of zwitterions in solution.

### The hydrated pathway

**CSD analysis.** In exploring the second hypothesis that a hydrated dimer provides a transition state for the assembly and crystallisation of  $\beta$ -*pABA* (hydrated pathway, black in Fig. 3) we first searched the CSD for crystal structures of hydrates of derivatives of uncharged *oABA*, *mABA* and *pABA*. This, however, revealed only one crystal structure (SOGGUR, 5-amino-2,4,6-tri-iodoisophthalic acid monohydrate). In order to derive more meaningful CSD statistics of relevant hydrated motifs the problem was then divided into three searches *viz.*: i) hydrates of benzoic acid derivatives, ii) hydrates of aniline derivatives and iii) hydrates of compounds containing at least a carboxylic acid and an amine group. Because of the paucity of structures containing all the required chemistry (aromatic ring, amine, carboxylic acid and water), the searches were done independently whereby specific preferences of hydrogen bonds and aromatic interactions could be compared. Table 4 summarises the results.

Examining first the hydrogen bond motifs we see that for hydrates of benzoic acid derivatives, the acid–acid dimer interaction is common, being found completely unsolvated in 18% of structures, solvated through one molecule of water only in 9% and solvated through two water molecules in

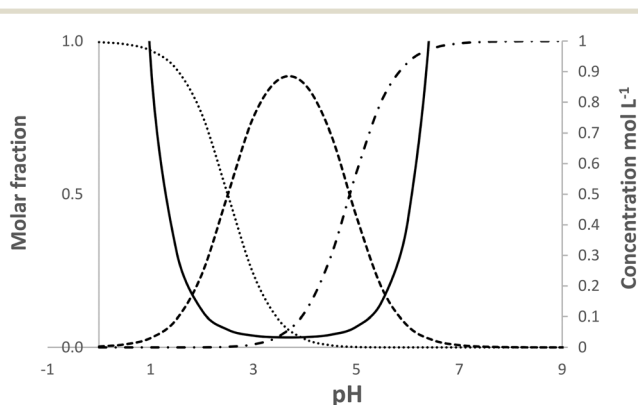


Fig. 5 Calculated speciation and solubility at 25 °C for *pABA* in water in the pH range 0 to 9. Solid line solubility, ...protonated species, -.-.- deprotonated species, ---- neutral species.



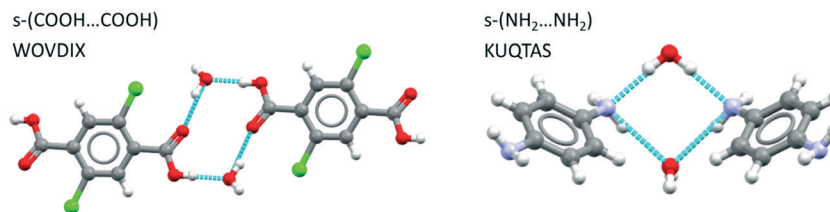


Fig. 6 Examples of solvated acid-acid dimers (left) and amine-amine dimers (right).

14%. The latter yields the desired tetramer, an example of which is displayed in Fig. 6(left) for WOVDIX. For aniline derivatives, the unsolvated amine-amine interaction is found in only 2% of structures (*i.e.* it is considerably less common than the acid dimer) while the fully hydrated tetramer is found in 8% as illustrated in Fig. 6 (right) for KUQTAS (*p*-phenylenediamine dihydrate). For hydrates of compounds with a carboxylic acid and an amine group (amine-X-acid), we found that the most common interaction is the unsolvated acid...amine dimer (13%), followed by the unsolvated acid...acid dimer (7%) and the solvated acid...acid tetramer (3%) similar to Fig. 6 (left).

As an adjunct to identifying the hydrated H-bonding motifs we further analysed the benzoic acid and aniline hydrate search results for aromatic stacks. 60–68% of these structures had aromatic stacking related by either translation or inversion. Remarkably, the aromatic stacking related by inversion comprised over 50% of the data in both groups. This is an important observation since stacks related by inversion are rare in unsolvated crystals of aminobenzoic acids as seen in Tables 2 and 3.

The overall conclusion from these CSD searches is that for hydrated structures aromatic interactions, both the inverted and translated stacks, are common whereas for the hydrogen bonds, the  $\alpha$  *p*ABA acid dimers are vastly more common than the acid-amine interactions. This hints at the stability of hydrated inverted stacks making them plausible precursors of the  $\beta$ -form of *p*ABA as per the scheme of Fig. 3.

**CSP – structures and motifs of hypothetical *p*ABA hydrates.** Following the CSD analysis and prior to considering an experimental search for *p*ABA hydrates, we considered the results and implication of CSP. Here we performed and analysed crystal structure prediction calculations of 1:1 and 1:2 *p*ABA:water hydrates (see ESI† section 4 for structural landscapes). Analysis of motifs was done using the CSD motif analyser and searches were performed for both the characteristic  $\alpha$  and  $\beta$  *p*ABA motifs and water-*p*ABA interactions. The results of these are summarised in Table 5. For the *p*ABA motifs, the  $R_2^2(8)$  acid dimer typical of  $\alpha$  *p*ABA is considerably more common (68 and 31% in 1:1 and 1:2 hydrate landscapes respectively) than the COOH...NH<sub>2</sub> motif typical of  $\beta$  *p*ABA (9 and 1% for the 1:1 and 1:2 respectively). The  $\alpha$  *p*ABA aromatic stacking is also more common than the  $\beta$  *p*ABA stacking (31 and 16% for 1:1 and 1:2 *versus* 2 and 3%).

Overall, the results show that both the aromatic stacking with translation symmetry and the unsolvated acid-acid dimer are present as motifs in the majority of the most stable predictions. These motifs are characteristic of the  $\alpha$  form. The water mostly hydrates the amino groups in the 1:1 hydrates by forming amino-amino hydrated tetramers and in the 1:2 hydrates by forming more complex water ring motifs (Fig. 7).

Analysis of the water...*p*ABA interactions show that one of the most abundant of those is the NH<sub>2</sub>(donor) ...water(acceptor) interaction in all 1:1 and 1:2 solvates. This is perhaps unsurprising since in all *p*ABA polymorphs there is always one –NH group which remains unable to hydrogen bond. Interestingly, the proportion of acid (donor) ...water (acceptor) interactions decreases dramatically from 71% to 30% in going from the 1:2 to 1:1 structures.

**Is a hydrate structure plausible?** Having explored the potential motifs within the CSP landscape we now consider whether or not a hydrate may actually exist. Using the most stable structures from the CSP of 1:1 and 1:2 *p*ABA:water hydrates, it was possible to calculate the free energy of hydrate formation for *p*ABA using the solvation model recently developed by Cruz-Cabeza *et al.*<sup>36</sup> The model requires the computation of lattice energies using DFT-d, together with estimates of the entropy penalty of hydration using the enthalpy of fusion of ice. For the latter, sophisticated DFT-d methods<sup>37,38</sup> were used. Table 6

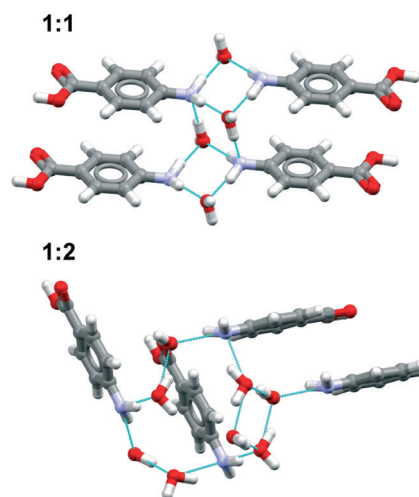


Fig. 7 Water hydrates the amino group in *p*ABA 1:1 and 1:2 predicted hydrate structures.



summarises the free energy results, showing that formation of these hydrates will not occur spontaneously, the calculated  $\Delta G$  being positive.

Interestingly, application of the solvate prediction tool to further explore the likelihood of hydrate formation (based on the CSD) confirms this result, predicting that the formation of a *p*ABA hydrate is highly unlikely with a hydrate prediction probability score of 0.094.

Overall, we are left to conclude that the existence of a *p*ABA hydrate is very unlikely. If this is the case then, taken together with the discussion concerning zwitterions, it seems that neither scheme of Fig. 3 is amenable to experimental verification. Notwithstanding this, the next section considers the more general potential for the experimental isolation of *p*ABA hydrates and solvates in order to see what further insights might be obtained.

### The experimental search for solvated forms of *p*-aminocarboxylic acids

In the case of our experimental search for hydrates of *p*ABA or one of its derivatives we describe here how the CSD was used to inform our choice of target solutes and solvents.

#### The fluorination trick

During the many CSD searches performed over the course of this work, we made an intriguing observation that fluorinated analogues of carboxylic acids crystallise as hydrates more often than non-fluorinated analogues. Thus, Table 7 summarises such data for acetic, succinic and terephthalic acid analogues. Given this finding, we decided to use 4-amino-2,3,5,6-tetrafluorobenzoic acid (pF-*p*ABA) alongside *p*ABA in an experimental quest for hydrates.

#### Choice of solvent

In an attempt to make the best choice of crystallisation solvents for isolating hydrates, we turned again to our CSD search of carboxylic acids (Table 4) crystallising either as hydrates or anhydrous forms. Analysis of structures for which

information on the crystallisation solvent was available (Table 8) showed that acetone:water (35%), ethanol:water (31%) and methanol:water (21%) were most likely to result in hydrates. However, the data set is rather small (especially for the acetone:water data) and hence the results are of limited statistical significance. We notice that the probability of crystallising a hydrate is higher in the mixed solvents than in water, although this is most likely due to the organic component being added to increase the solubility of the target material. Accordingly we decided to use ethanol:water, methanol:water and acetone:water as our solvent mixtures, together with dioxane:water since previous literature reports suggest an as yet uncharacterised *p*ABA form obtained from dioxane<sup>39</sup> additionally high-pressure crystallisations were performed for *p*ABA in water.<sup>13</sup>

### Crystallisation outcomes

As predicted, none of these experiments, including the high pressure, yielded a hydrate. However, four new solvates were obtained: a 2:1 *p*ABA:acetone, a 1.5:1 *p*ABA:dioxane, 1:2 pF-*p*ABA:acetone and a 1:2 pF-*p*ABA:dioxane.

**New solvates of *p*ABA and pF-*p*ABA.** The main motifs found in these new solvates of *p*ABA and pF-*p*ABA are visualised in Fig. 8. In the acetone solvates (Fig. 8a and b), both *p*ABA and pF-*p*ABA display carboxylic acid dimers typical of the  $\alpha$  form. In *p*ABA, the acetone solvates only half of the *p*ABA molecules through one  $\text{NH}\cdots\text{O}=\text{(acetone)}$  interaction whereas in pF-*p*ABA, all pF-*p*ABA molecules are solvated with two  $\text{NH}\cdots\text{O}=\text{(acetone)}$  interactions per molecule. These acetone solvates show similar interactions to a *p*ABA: nitromethane solvate reported previously (XECTOR).<sup>40</sup>

The dioxane solvates (Fig. 8c and d) show significant differences. *p*ABA:dioxane is similar to the *p*ABA:acetone and *p*ABA:nitromethane solvates with the carboxylic acid dimers and the dioxane solvating the amino group through one  $\text{NH}\cdots\text{O}(\text{dioxane})$  interaction. However, in these dioxane solvates, all *p*ABA molecules are solvated unlike the acetates where it is only half of them. For pF-*p*ABA:

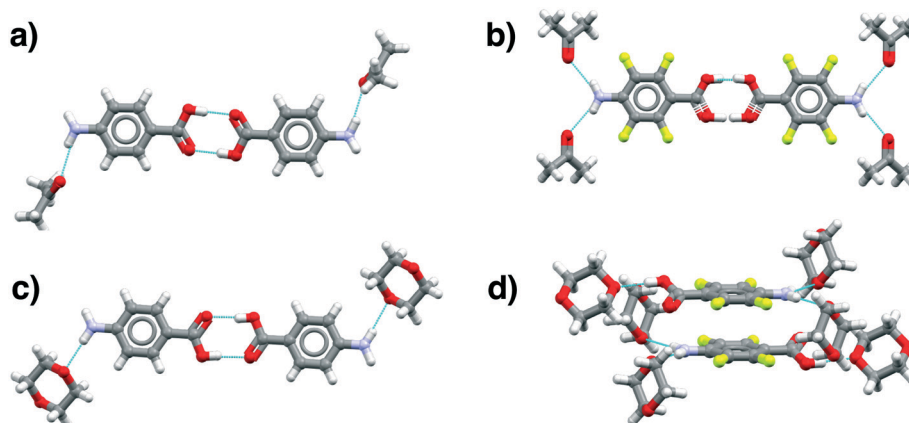


Fig. 8 Main motifs found in the *p*ABA (a and c) and pF-*p*ABA (b and d) solvates with acetone (a and b) and dioxane (c and d). See ESI† section 1 for CSD deposition numbers of these structures.



dioxane 1:2 solvate, the higher proportion of solvent to pF-*p*ABA molecules results in a full solvation of pF-*p*ABA not only around the amino group but also the carboxylic acid group. Most intriguingly, the only remaining interaction between pF-*p*ABA molecules in this structure is a stacked dimer in which the molecules are related by a centre of inversion (Fig. 8d).

Thus, our experiments confirm the predictions that a hydrate form of *p*ABA is highly unlikely. Further, they show that when *p*ABA is solvated that the acid dimer remains intact with solvation favoured at the amino moiety. Only in pF-*p*ABA is the solvent able to break the dimer with consequent formation of stacks related by inversion.

## Discussion and conclusions

Extensive use of the CSD together with CSP and significant numbers of targeted crystallisation experiments have failed to produce evidence for any form of solution phase molecular assembly, involving solute and/or solvent, that might be considered as a transition state in the nucleation of  $\beta$  *p*ABA. The study has confirmed that the motifs found in  $\beta$  *p*ABA are rare in the CSD, in the new structures of *p*ABA derivatives reported in this paper and in CSP of both *p*ABA reported previously and *p*ABA hydrates reported here. In fact, to create a crystal packing free from the acid dimer is indeed extremely difficult – 5 out of 6 of our new solvated structures are dimer based. However, the  $\beta$  motif is found in zwitterionic *m*ABA and *o*ABA structures and there is some tentative suggestion that a link may exist between the proportion of zwitterions in solution and the appearance of  $\beta$  but this cannot be confirmed experimentally. In terms of a solvated pathway, stable hydrates were not predicted and, indeed, we were unable to isolate a hydrated form. The dioxane solvate is alone in reflecting any element of the  $\beta$  structure, having no acid dimers and with phenyl rings related by inversion. This marries well with the fact that inverted stacks are more commonly observed in hydrates of benzoic acids than in anhydrous forms. This evidence supports the view that aromatic stacking is the first self-assembled interaction leading to the creation of molecular clusters and eventually crystal nuclei.<sup>12</sup> The subsequent formation of the acid dimers may then be solvent dependant, related to the extent of pre-dimerisation in solution. Finally, desolvation of the amino group takes place. Of course this aromatic stacking may lead to molecules and hence dimers either related by inversion or by translation. Dimers related by translation are in the correct geometry to further assemble through the acid–acid interaction resulting in  $\alpha$  *p*ABA. Dimers related by inversion would either need to re-arrange before forming the  $\alpha$  structure (pF-*p*ABA: dioxane desolvates directly to the structure of pF- $\alpha$  *p*ABA suggesting that this may be facile) or they could develop directly into the  $\beta$  polymorph. However,  $\beta$  appears only under very specific sets of conditions of low supersaturations and in aqueous solution so we may assume that it is here that dimers related by inversion can

successfully compete. Of course, under most conditions of solvent and supersaturation a cluster comprising stacked acid dimers related by translation wins out (giving the  $\alpha$  polymorph), growing much faster than a cluster of molecules related by inversion, which will need to grow *via* COOH $\cdots$ NH<sub>2</sub> chains. This conclusion is supported by our measurement of the relative rates of growth of  $\alpha$  and  $\beta$  crystals, which show that  $\alpha$  wins out by about 2 orders of magnitude.<sup>18</sup> A corollary to this is our previous demonstration that only by disrupting the translated stacks with an additive it is possible to inhibit their growth and thus prevent nucleation of the  $\alpha$  structure. This enables  $\beta$  to appear consistently.<sup>41</sup> Of course this line of argument parallels the assumptions made in the significant work of Weissbuch *et al.*<sup>42</sup> that ‘...our approach rests on a working hypothesis that in supersaturated solutions the molecules assemble to form coexisting clusters adopting a variety of shapes and arrangements, some of which resemble the structure of the macroscopic crystals into which they eventually develop.’ In the case of *p*ABA the outcome of the development of these clusters leads to the appearance of the  $\alpha$  polymorph with the exception of its crystallisation from water where  $\beta$  can appear at low supersaturations. We may now confirm that the role of water here is not to provide a solution mediated pathway to  $\beta$ . Instead, it is the unusually high value of the *p*ABA/water interfacial tension (4.83 mJ m<sup>−2</sup> compared to 1–2.8 mJ m<sup>−2</sup> in organic solvents), that creates a nucleation and growth dead zone, at low supersaturations, where  $\alpha$  clusters cannot grow thus giving time for  $\beta$  to appear.<sup>18,43</sup>

## Conflicts of interest

There are no conflicts to declare.

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