



Cite this: *CrystEngComm*, 2018, 20, 7308

Exploring polymorphism and stoichiometric diversity in naproxen/proline cocrystals†

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We present naproxen/proline cocrystals discovered when combining enantiopure and racemic naproxen and proline. Using liquid-assisted grinding as the main method to explore the variety of crystal forms in this system, we found 17 cocrystals, of which the structures of only four of them were previously known. The naproxen/proline system exhibited multiple polymorphs of 1:1 stoichiometry as well as more rare cocrystals with 1:2 and 2:3 stoichiometries, two cocrystal hydrates and one cocrystal solvate. *In situ* ball-milling, used to monitor liquid-assisted grinding reactions, revealed that the solvent dictates the reaction intermediates even if the final reaction product stays the same. Synchrotron X-ray diffraction data collected *in situ* upon heating allowed us to monitor directly the phase changes upon heating and gave access to pure diffraction patterns of several cocrystals, thus enabling their structure determination from powder X-ray diffraction data; this method also confirmed the formation of a conglomerate in the *RS*-naproxen/*DL*-proline system. Proline in cocrystals kept its ability to form charge-assisted head-to-tail N-H...O hydrogen bonds, typical of pure crystalline amino acids, thus increasing the percentage of strong charge-assisted interactions in the structure and consequently providing some of the cocrystals with higher melting points as compared to pure naproxen. The majority of drugs are chiral, and hence, these data are of importance to the pharmaceutical industry as they provide insight into the challenges of chiral cocrystallization.

Received 9th August 2018,
Accepted 15th October 2018

DOI: 10.1039/c8ce01338a

rsc.li/crystengcomm

Introduction

Multicomponent crystalline systems had been known long before the term “cocrystal” gained its popularity.^{1,2} The field of cocrystallization and the concept of crystal engineering has especially flourished in the last decades. Engineering cocrystals has become a widespread solution to various structure–property related problems, *e.g.*, improving the therapeutic performance of drugs in the pharmaceutical industry^{3–5} or

tuning the properties of thermo- and photochromic materials, molecular semiconductors and optical materials.^{6–10}

Chiral cocrystals are of special importance in the pharmaceutical industry as a substantial part of drugs are chiral.¹¹ Knowing how the enantiomeric or racemic forms of the same drug would respond to cocrystallization with chiral and/or non-chiral coformers, *i.e.*, the differences in both the structure and properties of those cocrystals, their ability to cocrystallize, and production related issues, is essential in developing robust and effective crystallization processes. There have been a number of studies in this area. For instance, caffeine and theophylline were found to respond differently to cocrystallization with *DL*- and *D*-tartaric acid.^{12,13} Enantiospecific behavior, when only one of the enantiomers was found to form a cocrystal with a certain chiral coformer, was reported for various systems, *e.g.*, the stanolone/tartaric acid,¹⁴ cyclohexanediol/tartaric acid,¹⁵ levetiracetam/mandelic acid and levetiracetam/tartaric acid,¹⁶ and naproxen/tyrosine¹⁷ systems. This property of chiral cocrystals was used to develop alternative chiral resolution techniques based on cocrystallization.^{18–24}

The majority of amino acids are chiral. Their use in the pharmaceutical industry as potential coformers can be

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† Electronic supplementary information (ESI) available: Experimental details on structure determination along with additional experimental details of sample preparation and sample analysis, including DSC and PXRD data. The CCDC deposition numbers of the reported structures are 1861015–1861028. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c8ce01338a

advantageous as they are natural to the body and thus present significantly lower risks in comparison with other coformers. Amino acids are zwitterionic in the crystalline state and tend to form head-to-tail charge-assisted hydrogen-bonded chains^{25–27} that are also observed in cocrystals;^{17,28} this structural feature of amino acids may help to improve the API's properties, for instance by increasing stability as the percentage of strong interactions in the structure grows.²⁹

Proline is special as, unlike other amino acids, its amino group is found within the pyrrolidine ring. It showed a good ability to cocrystallize with various compounds for multiple applications, *i.e.*, to alter the physicochemical properties of drugs,^{30–35} for chiral resolution,^{36–38} to develop an amino acid derived semiconductor,³⁹ *etc.* Proline has also been used for ionic cocrystal formation to investigate the possibilities of chiral resolution in the solid state.⁴⁰

In our recent work, we combined enantiopure and racemic forms of flurbiprofen and proline and, with the help of various state-of-the-art characterization methods, identified 17 cocrystals.⁴¹ In this work, we chose another model compound, naproxen, to study the cocrystallization of chiral coformers. Naproxen is a nonsteroidal anti-inflammatory drug poorly soluble in water; several cocrystals of naproxen have already been developed with the purpose of modifying its properties.⁴² Its cocrystallization with amino acids may suggest an alternative way to improve its therapeutic performance. In one of our previous studies, naproxen has already shown its ability to cocrystallize with amino acids, such as alanine, tryptophan, tyrosine,¹⁷ and arginine.⁴³ In addition, several cocrystals of naproxen (nprx) and proline (pro) have been previously reported.²⁸

The naproxen/proline system was selected because by analogy with our previous work on flurbiprofen we suspected that the system might exhibit many more cocrystal forms than the Cambridge Structural Database reports. Analyzing a large variety of cocrystal forms on a limited set of coformers enables an insight not only into various aspects of chirality in cocrystallization but also how it may be related with polymorphism and stoichiometric diversity. In this work, applying a combination of modern characterization methods, including synchrotron X-ray diffraction under various conditions, we discovered multiple previously unknown nprx/pro cocrystal phases. The nprx/pro system, with its 17 cocrystals (including two cocrystal hydrates and one cocrystal solvate) and one conglomerate, is another fascinating example of complexity that can be reached in a chiral system when using pro as a coformer.

Experimental

The experimental procedures are described below, except the procedure for the production of single crystals and structure determination from single-crystal and powder X-ray diffraction data which are given in the ESI.†

Materials

All the compounds for this work were purchased from Sigma-Aldrich and used without further purification. The reference diffraction patterns of the initial compounds were simulated using the Mercury software⁴⁴ from the corresponding structures found in the Cambridge Structural Database: *S*-nprx (CCDC COYRUD,⁴⁵ COYRUD01,⁴⁶ COYRUD11,⁴⁷ and COYRUD12,⁴⁸ COYRUD13,⁴⁹ COYRUD14⁵⁰), *RS*-nprx (CCDC PAPTUX⁵¹ and PAPTUX01⁵²), *L*-pro (CCDC PROLIN,⁵³ PROLIN02,⁴¹ and PROLIN03⁴¹), *L*-pro monohydrate (CCDC RUWGEV⁵⁴), *DL*-pro (CCDC QANRUT⁵⁵ and QANRUT01⁵⁶), and *DL*-pro monohydrate (CCDC DLPROM01⁵⁷).

Laboratory liquid-assisted grinding (LAG) and laboratory powder X-ray diffraction analysis

Liquid-assisted grinding in the presence of five different solvents, methanol (MeOH), ethanol (EtOH), isopropanol (ISPN), acetonitrile (ACN), and water, was performed for five combinations of enantiopure and racemic nprx and pro: *S*-nprx/*L*-pro, *S*-nprx/*D*-pro, *S*-nprx/*DL*-pro, *RS*-nprx/*L*-pro, and *RS*-nprx/*DL*-pro. The compounds were ground in 1:1, 1:2, 2:3, and 2:1 ratios. To prepare each sample, 50–100 mg of total powder were placed into a 2 mL Eppendorf tube and 2–10 μ L of solvent were added along with 5–6 stainless steel 2 mm balls to ensure grinding; the Eppendorf tubes were placed into a Retsch MM400 mixer mill for 60 min at a frequency of 30 Hz.

The resultant powders were analyzed by powder X-ray diffraction (PXRD) and CuK α radiation using a Siemens D5000 diffractometer (2θ was scanned from 2° to 50° with a step of 0.02°) or an X'Pert PRO PANalytical diffractometer (2θ scanned from 4° to 50° with a step of 0.017°). Collected diffraction patterns were analyzed by comparison with those of initial pure compounds and other known cocrystal phases in order to identify whether anything new formed.

Some samples were chosen for further analysis by other methods. All LAG experiments, their outcome, and characterization methods are summarized in Tables S4.1, S5.1, S6.1, S7.1, S8.1, and S9.1.†

In situ synchrotron variable-temperature X-ray diffraction data collection

Selected powders obtained by liquid-assisted grinding were packed into 0.5 mm glass capillaries and measured upon heating at a rate of 2 °C min^{−1}. All powder diffraction data, except data from an *in situ* experiment for the 1:1 *RS*-nprx/*L*-pro/H₂O (sample 17 in Table S7.1†) and 1:1 *RS*-nprx/*DL*-pro/H₂O (sample 17 in Table S8.1†), were collected using a synchrotron radiation source: MS-X04SA beam line at the Swiss Light Source (SLS) (PSI, Switzerland) equipped with the 1D microstrip detector MYTHEN II. The measurements were taken at λ = 0.77663, 0.62173, and 0.708 Å with a step of 0.0036°. The wavelength was calibrated using a standard NIST 640d Si sample. The diffraction patterns were collected during continuous heating; the acquisition time for each

powder pattern was 40 s. Selected diffraction patterns were used for structure determination from X-ray powder data. The 1 : 1 *RS*-npx/*L*-pro/ H_2O (sample 17 in Table S7.1[†]) and 1 : 1 *RS*-npx/*DL*-pro/ H_2O (sample 17 in Table S8.1[†]) were measured at the Swiss-Norwegian beamline BM1A at the European Synchrotron Radiation Facility (ESRF) (Grenoble, France), using a PILATUS 2M hybrid pixel detector at a wavelength of 0.78487 Å. The diffraction patterns were collected as well during continuous heating and the acquisition time for each frame was 30 s.

In situ ball-milling synchrotron X-ray diffraction data collection

In situ ball-milling X-ray diffraction experiments were performed at the μSpot beamline (BESSY II, Helmholtz Centre Berlin for Materials and Energy, Germany) using a Pulverisette 23 (Fritsch, Germany) ball mill and Perspex jars; the frequency was 50 Hz. The experimental setup was the same as that in ref. 58, where it is described in detail, except Raman spectroscopy was not used. The compounds were mixed and the solvent and 3 stainless steel 5 mm balls were added right before the experiment. Details for each experiment (duration of milling, amount of solvent, amounts of initial compounds) are summarized in Table S3.1[†]. A beam diameter of 100 μm at a photon flux of $1 \times 10^9 \text{ s}^{-1}$ at a ring current of 100 mA was used. The experiments were performed at a wavelength of 1.000 Å using a double crystal monochromator (Si (111)). The spot size on the sample was 200 μm . Scattered intensities were collected with a two-dimensional X-ray detector (MarMosaic, CCD 3072×3072 pixels, pixel size 73 μm).⁵⁹ Measurements were carried out every 30 s with a readout time of 3 or 4 s between two measurements. The occasionally observed doubling of reflections is caused by diffraction of two different areas in the reaction jar due to differences in the distance between the detector and the sample. The obtained two-dimensional diffraction images were integrated (2θ vs. intensity) using the program Fit2D.⁶⁰ Diffraction patterns were background corrected and plotted as a 2D film representation using the Powder3D program.⁶¹

Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA)

DSC curves were measured using a DSC 821 Mettler Toledo instrument. Prior to measurements, the instrument was calibrated using indium. Standard 40 μL aluminum crucibles were used. The heating rate was 2°C min^{-1} over the range from 25 to 200–250 $^\circ\text{C}$. All experiments were carried out under a nitrogen atmosphere (a flow rate of 50 ml min^{-1}). DSC curves of the selected samples are given in the ESI.[†]

DSC data were used to select multiphase samples (samples from the powder X-ray diffraction analysis that were suspected to contain multiple phases) and to determine melting points when possible. Samples with complicated DSC curve shapes were further analyzed *in situ* using variable-temperature synchrotron radiation in order to identify and directly monitor phase changes upon heating. It should be

noted that direct matching of the DSC and *in situ* synchrotron PXRD data may not be always reliable due to different experimental setups; however, *in situ* synchrotron PXRD data provide a more profound view and might help in interpretation of the DSC data.

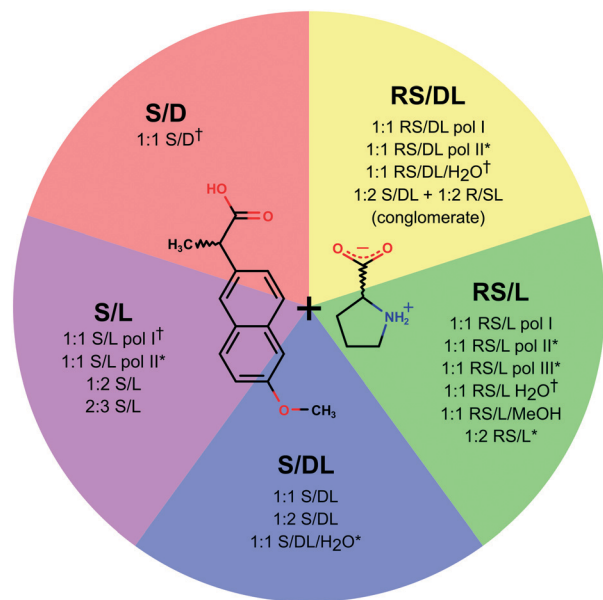
The TGA curve was measured in order to determine the number of water molecules in the asymmetric unit of the 1 : 1 *S*/*DL*/ H_2O cocrystal hydrate (sample 17 in Table S9.1[†]) needed to solve the structure of this phase from PXRD. The measurement was performed in the temperature range from 25 to 400 $^\circ\text{C}$ and at a heating rate of 5°C min^{-1} using a Mettler Toledo TGA/DSC 3+ instrument; the obtained TGA curve is given in Fig. S9.3.6[†]

Results and discussion

We studied five combinations of enantiopure and racemic naproxen and proline: *S*-npx/*L*-pro (= *R*-npx/*D*-pro), *S*-npx/*D*-pro (= *R*-npx/*L*-pro), *S*-npx/*DL*-pro (= *R*-npx/*DL*-pro), *RS*-npx/*L*-pro (= *RS*-npx/*D*-pro), and *RS*-npx/*DL*-pro. The main approach to explore cocrystal phases was liquid-assisted grinding (LAG); solution cocrystallization was used only to produce single crystals. The advantages of LAG were outlined in our previous work,⁴¹ where this method proved highly efficient. LAG was performed in the presence of the following five solvents and varying the ratio of the initial compounds: methanol (MeOH), ethanol (EtOH), isopropanol (ISPN), acetonitrile (ACN), and water. The solvents were chosen so that at least one of the initial compounds will be well soluble: npx is relatively well soluble in all the solvents except water, whereas pro exhibits decent solubility only in MeOH, EtOH, and water. The powders obtained after LAG reactions were first subjected to simple laboratory PXRD analysis in order to identify known phases; then, DSC analysis was performed for selected samples, representing different compositions, in order to check for both the purity of the samples and any occurring phase transformations as well as to determine the melting points if possible. The samples that exhibited complicated DSC curves with multiple peaks were further subjected to variable-temperature *in situ* synchrotron X-ray diffraction analysis to gain a direct insight into phase changes. The structures of those cocrystal phases for which we failed to grow sufficiently big single crystals for single-crystal X-ray diffraction analysis were solved from powder X-ray diffraction data collected using synchrotron radiation. Scheme 1 gives an overview of the results. As all the combinations, except *S*/*D*, resulted in multiple cocrystal phases, we decided to perform *in situ* ball-milling experiments monitored by synchrotron radiation in order to get a deeper insight into mechanochemical reactions. Each combination is discussed below in detail. The structures of the obtained cocrystals are discussed in the Structural analysis section.

S-Naproxen/*L*-proline (*S*/*L*)

The *S*/*L* system yielded the richest variety of cocrystals, including cocrystals with a different stoichiometry. All the



Scheme 1 A schematic summary of all the found cocrystals for the five nprx/pro combinations. The structures of the cocrystals were determined by single-crystal X-ray diffraction analysis, except those marked as ‘†’ and ‘*’. The structures marked as ‘†’ were found from powder X-ray diffraction data and those marked as ‘*’ were already known from literature.

cocrystals whose structures were determined can be found in Scheme 1.

Preliminary laboratory LAG experiments using a series of solvents (MeOH, EtOH, ISPN, and ACN) did not allow us to draw any reliable conclusions about whether the reaction outcome is solvent-dependent: all the phases seemed to have chances to emerge. Table S4.1[†] summarizes the outcomes of the laboratory LAG experiments. From this table we see that among the 1:1 forms, 1:1 S/L pol I (CCDC REF FEVZUD²⁸) seemed to emerge more often in MeOH- and EtOH-LAG reactions, whereas ACN- and ISPN-LAG resulted mainly in the 1:1 S/L pol II. However, some samples contained both polymorphs in varied amounts. LAG reactions when pro was taken in an excess amount (1:2 and 2:3 nprx:pro ratios) showed the formation of the 2:3 S/L cocrystal, whereas the 2:1 nprx:pro ratio yielded mainly 1:1 S/L polymorphs. The 1:2 S/L cocrystal that was crystallized from solution during our attempts to obtain single crystals was detected only in 1:2 water-LAG and 2:3 ISPN-LAG (see samples 12 and 18 in Table S4.1[†]) and not in any other LAG reactions, even if the initial ratio of the components was 1:2 (see samples 2, 6, 10, and 14 in Table S4.1[†]). As can be seen from Table S4.1[†], most of the laboratory LAG experiments led to product mixtures, which means that the cocrystal phases kinetically compete with one another. We performed *in situ* ball-milling experiments in order to gain insight into the grinding process and directly monitor the phase changes in the course of the reaction. The results are presented in Fig. 1.

All the *in situ* monitored LAG reactions finally led to the 1:1 S/L pol II phase, but the pathway differed (samples 1–4

in Table S3.1[†]). In MeOH- and EtOH-LAG, a mixture of the 1:1 S/L pol I and 2:3 S/L preceded the formation of the 1:1 S/L pol II (Fig. 1a and b, S3.1.1 and S3.1.2[†]) and disappeared by the 6th minute of reaction, thus indicating that the former phases are more kinetically favored under these conditions but not stable enough to withstand further grinding. In ISPN- and ACN-LAG (Fig. 1c and d), we saw an unknown phase emerging in the beginning and then disappearing during the course of the reaction (we called it the unknown phase I), which thus was as well less stable but kinetically faster than 1:1 S/L pol II under the grinding conditions. Hence, *in situ* ball-milling experiments showed that varying the solvent affects the reaction pathway by revealing the formation of different phases that precede the main 1:1 S/L pol II phase depending on the solvent used – the result that was not apparent in the laboratory experiments.

In situ ball-milling reaction outcomes differed from those obtained by 1:1 laboratory LAG: in laboratory experiments (1:1 ratio) both 1:1 S/L pol I and 1:1 S/L pol II had chances to emerge, unlike the *in situ* ball-milled powders that contained only pol II as the final reaction product, no matter which solvent was used. This result can be explained by the fact that grinding reactions may be very sensitive to the ball-milling setup (the amount of powder, the diameter of balls, the volume of liquid added per total amount of powder, the jar's configuration and the material the jar is made of, etc.).

However, despite the difference, we can still make some parallels between the laboratory and *in situ* ball-milling data. For instance, the precedence of the 1:1 S/L pol I to the emergence of the 1:1 S/L pol II in MeOH-LAG (Fig. 1a) indicates that pol I can be the kinetic form, whereas pol II is the thermodynamic form, which explains why in some 1:1 laboratory grinding reactions we obtained one or the other or both polymorphs at the same time, depending on the conditions. According to Ostwald's rule, the least stable polymorph should crystallize first, which explains why from solution crystallization we always obtained pol I and failed to obtain pol II.

DSC curves measured for selected samples (see Fig. S4.3.1–S4.3.5[†]) had complex shapes with multiple peaks. This is why we decided to measure variable-temperature *in situ* synchrotron PXRD for those samples in order to monitor phase changes upon heating.

The first example of a variable-temperature *in situ* experiment is presented in Fig. 2. The initial sample obtained by 1:1 ACN-LAG contained a mixture of two 1:1 polymorphs: pol I and pol II. Upon heating, the 1:1 S/L pol I disappeared, leaving only pol II in the sample. Upon further heating, the disappearance of the 1:1 S/L pol II was followed by the emergence of the 2:3 S/L.

The second example of a variable-temperature *in situ* experiment is presented in Fig. 3. The sample obtained by 1:1 EtOH-LAG reaction initially contained only 1:1 S/L pol I. Upon heating, the 1:1 S/L pol I phase disappeared followed

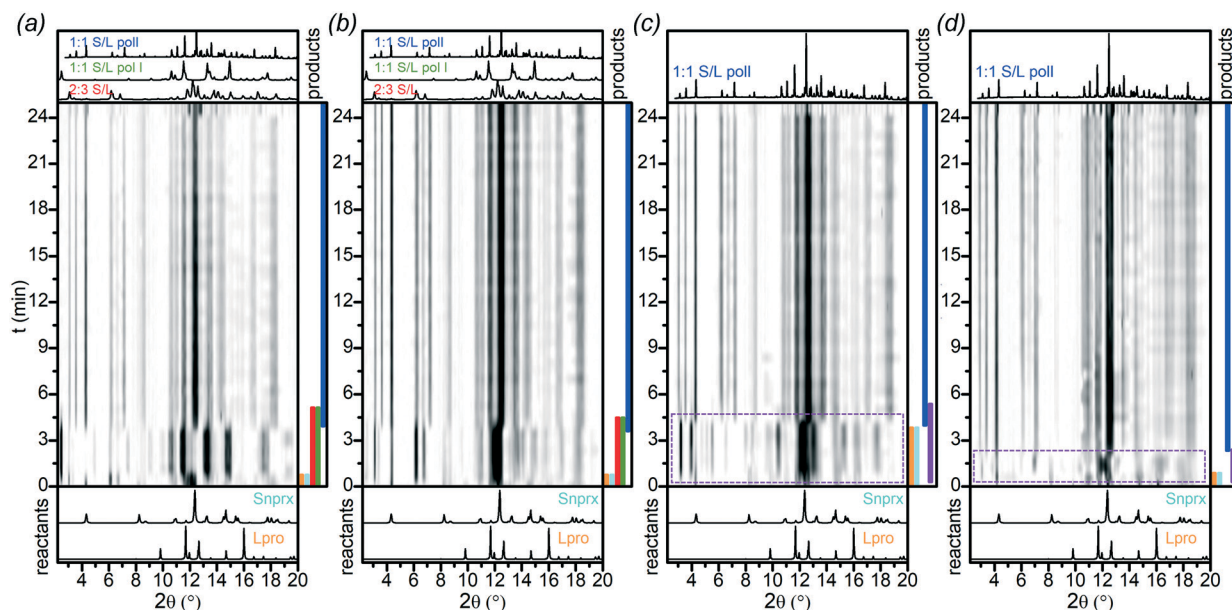


Fig. 1 *In situ* ball-milling X-ray diffraction patterns for the *S/L* system, $\lambda = 1 \text{ \AA}$, 1:1 ratio: (a) MeOH-, (b) EtOH-, (c) ISPN-, and (d) ACN-LAG. Colored bands on the right side of each image represent the presence of phases and are shown only for illustrative purposes; the area outlined by purple in (c) and (d) shows the presence of the unknown phase I.

by the emergence of the 2:3 *S/L* and unknown phase II. The unknown phase II disappears first upon further heating.

From the *in situ* variable-temperature PXRD data, it follows that the 2:3 *S/L* phase must have the highest melting point among the *S/L* cocrystal phases as it is the last phase registered before complete melting (Fig. 2, 3 and S4.1.1†). The 1:1 *S/L* pol I cocrystal should have a lower melting point than 1:1 *S/L* pol II as it disappears first upon heating. However, the exact determination of the melting points of both polymorphs was difficult due to the complexity of the DSC curves (Fig. S4.3.1–S4.3.3†).

The possibility of the formation of cocrystal hydrates was checked by performing LAG in the presence of water. No new phases were detected (see samples 17–19 in Table S4.1†).

In conclusion, for the *S/L* combination we identified multiple polymorphs and stoichiometrically diverse cocrystals (1:1, 1:1:2, and 2:3). *In situ* ball-milling revealed the presence of

intermediate phases that differed depending on the solvent used, even though the final reaction product was the same. Variable-temperature *in situ* PXRD provided insight into the stability of various forms upon heating, with the 1:1 *S/L* pol I being the least thermally stable, and the 2:3 *S/L* having the highest thermal stability among the *S/L* forms.

S-Naproxen/*D*-proline (*S/D*)

The *S/D* system turned out to be the simplest: only one 1:1 *S/D* cocrystal, whose structure has already been reported²⁸ (CCDC REF FEVZOX²⁸), was detected in our experiments (see Table S5.1† for the summary of performed experiments). Since preliminary laboratory experiments did not yield any new forms, we decided not to explore this combination any further. However, we accept the possibility that other cocrystal forms may emerge if screening conditions are

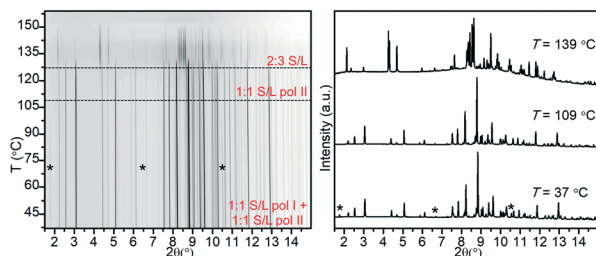


Fig. 2 PXRD patterns measured *in situ* upon heating (from 37 to 159 °C, 2 °C min⁻¹) for the *S/L* sample prepared by 1:1 ACN-LAG (sample 13 in Table S4.1†), $\lambda = 0.708 \text{ \AA}$: (left) film representation; (right) XRD patterns at selected temperatures: $T = 37 \text{ °C}$ – 1:1 *S/L* pol I and 1:1 *S/L* pol II; $T = 109 \text{ °C}$ – 1:1 *S/L* pol II; and $T = 139 \text{ °C}$ – 2:3 *S/L*.

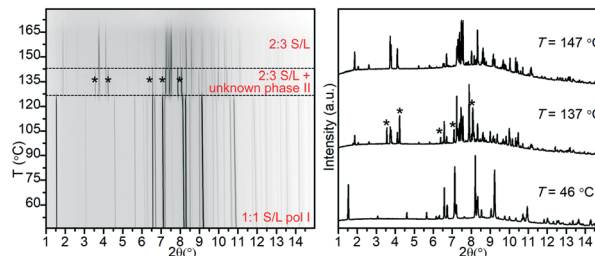


Fig. 3 PXRD patterns measured *in situ* upon heating (from 46 to 179 °C, 2 °C min⁻¹) for the *S/L* sample prepared by 1:1 EtOH-LAG (sample 5 in Table S4.1†), $\lambda = 0.62127 \text{ \AA}$: (left) film representation; (right) XRD patterns at selected temperatures: $T = 46 \text{ °C}$ – 1:1 *S/L* pol I; $T = 137 \text{ °C}$ – 2:3 *S/L* and unknown phase II; and $T = 147 \text{ °C}$ – 2:3 *S/L*.

changed, e.g., if other cocrystallization techniques are used, but this aspect goes beyond the scope of the present work.

S-Naproxen/DL-proline

The S/DL system yielded several forms: one cocrystal with the 1:1 stoichiometry, one with the 1:2 stoichiometry, and one 1:1 cocrystal hydrate (see Scheme 1). Laboratory LAG experiments (see Table S9.1†) showed that varying the solvent in LAG hardly affected the reaction outcome: MeOH-, EtOH-, ISPN-, and ACN-LAG reactions led to the same products: 1:1 LAG reactions resulted in 1:1 S/DL as the main product and both the 1:2 S/DL and 1:1 $S/DL/H_2O$ phases as impurities; 1:2 LAGs gave only 1:2 S/DL without significant amounts of impurities; in 2:1 LAGs, we observed mainly 1:1 S/DL and unreacted S-nprx; and finally 2:3 LAGs resulted in mixtures of the 1:1 S/DL and 1:2 S/DL phases.

These results were in agreement with results of the *in situ* ball-milling experiments (Fig. 4). All the *in situ* monitored reactions in the presence of the aforementioned four solvents led to (1:1 S/DL 1:2 S/DL) mixtures (samples 5–8 in Table S9.1†). In the MeOH-LAG the 1:2 S/DL and the 1:1 S/DL phases seem to start emerging approximately at the same time (Fig. 4a and S3.1.15†). In the EtOH-LAG sample, the 1:2 S/DL clearly starts emerging slightly earlier than the 1:1 S/DL (Fig. 4b and S3.1.16†). Finally, the ISPN- and ACN-LAG samples clearly show that the 1:2 S/DL emerges first and the 1:1 S/DL phase appears later (starting from the 5th–6th min of reaction) (Fig. 4c and d). These results indicate that the 1:2 form is more kinetically favorable than the 1:1 form. However, both the 1:2 S/DL and 1:1 S/DL are relatively stable and, therefore, once formed, do not undergo further conversion (or the rate of conversion is very low), which explains why the

final powders after grinding contain both these phases in significant amounts. As was shown in our previous work,⁴¹ the solvent can affect the reaction rate, which is why it takes longer for the initial reactants to react in ACN- and ISPN-LAG than in MeOH- and EtOH-LAG reactions: the initial reactants stay until the 5th–6th min in ACN- and ISPN-LAG reactions, whereas they completely disappear already by the 2nd and 4th min in MeOH- and EtOH-LAG, respectively. Performing reactions in the presence of various solvents to vary the reaction rate helps in establishing the order in which the reaction products emerge: in our particular case, slow ACN- and ISPN-LAGs showed most clearly that the 1:2 S/DL phase is more kinetically favored than the 1:1 S/DL under grinding conditions.

As many of the laboratory grinding experiments for this system yielded multiphase samples, we performed variable-temperature *in situ* synchrotron X-ray diffraction measurements for selected powders in order to establish relative stabilities of the phases and their behavior upon heating. All the selected samples behaved in a similar manner (samples 1, 9, 11, and 13 in Table S9.1†). Fig. 5 demonstrates one of the examples: a sample prepared by 1:1 MeOH-LAG, which initially contained a mixture of 1:1 S/DL , 1:2 S/DL , and 1:1 $S/DL/H_2O$ (other examples can be found in Fig. S9.1.1–S9.1.3†). Water can be easily taken from air or trace amounts of it can be present in the solvent. When the sample is subjected to heating, first the 1:1 $S/DL/H_2O$ impurity disappears followed by the disappearance of the 1:1 S/DL phase, which leaves pure 1:2 S/DL until it melts at around 170 °C, thereby showing that the latter is the most thermally stable among the S/DL phases.

Water-assisted grinding favors the formation of the 1:1 $S/DL/H_2O$ phase (samples 17–20 in Table S9.1†). As was mentioned above, the presence of this phase was also observed in

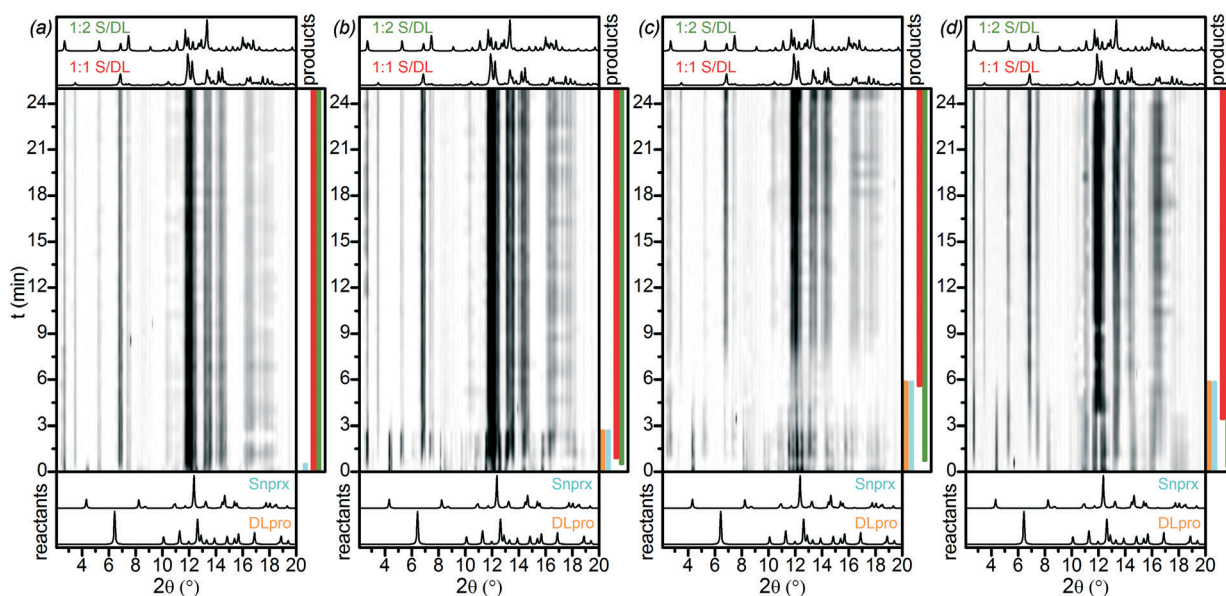


Fig. 4 *In situ* ball-milling X-ray diffraction patterns for the S/DL system, $\lambda = 1 \text{ \AA}$, 1:1 ratio: (a) MeOH-, (b) EtOH-, (c) ISPN-, and (d) ACN-LAG. Colored bands on the right side of each image represent the presence of phases and are shown only for illustrative purposes.

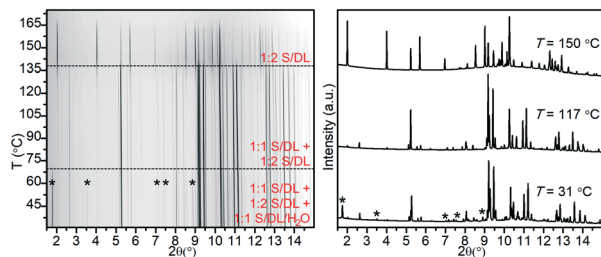


Fig. 5 PXRD patterns measured *in situ* upon heating (from 31 to 175 °C, 2 °C min⁻¹) for the *S/DL* sample prepared by 1:1 MeOH-LAG (sample 1 in Table S9.1†), $\lambda = 0.77663$ Å: (left) film representation; (right) XRD patterns at selected temperatures: $T = 31$ °C – 1:1 *S/DL*, 1:2 *S/DL*, and 1:1 *S/DL/H₂O* (marked as *); $T = 117$ °C – 1:1 *S/DL* and 1:2 *S/DL*; and $T = 150$ °C – 1:2 *S/DL*.

other laboratory LAG experiments (see Fig. S9.1.4† for the comparison of the 1:1 MeOH- and water-LAG reaction products).

In order to understand how the hydrated *S/DL* form will behave upon heating, we performed variable-temperature *in situ* synchrotron PXRD. We did not observe any new phases. Upon heating, the disappearance of the 1:1 *S/DL/H₂O* was followed by the emergence of a (1:1 + 1:2 *S/DL*) mixture, which upon further heating exhibited the same behavior as the other measured samples: the 1:1 *S/DL* phase melted before the 1:2 *S/DL* (Fig. 6).

We thus conclude that the *S/DL* system was also stoichiometrically diverse, showing 1:1 and 1:2 cocrystals. Water-LAG led to the formation of a cocrystal hydrate. *In situ* ball-milling experiments revealed that the 1:2 *S/DL* phase is more kinetically favored under these conditions and emerges before the 1:1 *S/DL* (the reaction rate depends on the solvent used). Finally, the *in situ* variable-temperature experiments showed that the 1:2 *S/DL* has the highest thermal stability and melts the last among the *S/DL* phases.

RS-Naproxen/L-proline (RS/L)

As shown in Scheme 1, we found 6 cocrystals for the *RS/L* system: three 1:1 polymorphs, one 1:2 cocrystal, one 1:1 cocrystal hydrate, and one 1:1 cocrystal methanol solvate.

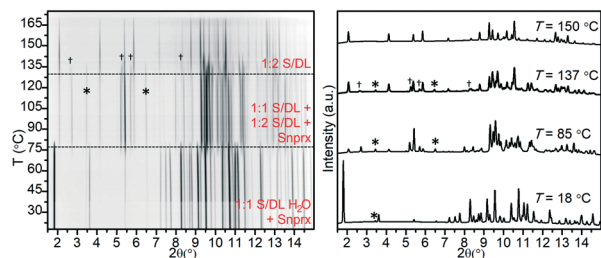


Fig. 6 PXRD patterns measured *in situ* upon heating (from 18 to 175 °C, 2 °C min⁻¹) for the *S/DL* sample prepared by 1:1 water-LAG (sample 17 in Table S9.1†), $\lambda = 0.798$ Å: (left) film representation; (right) XRD patterns at selected temperatures: $T = 18$ °C – 1:1 *S/DL/H₂O* and *S*-nprx (marked as *); $T = 85$ °C – 1:1 *S/DL*, 1:2 *S/DL*, and *S*-nprx; and $T = 150$ °C – 1:2 *S/DL*.

The analysis of laboratory LAG experiments showed that the reaction outcome is solvent-dependent: 1:1 MeOH-, EtOH-, and ISPN-LAG resulted in the 1:1 *RS/L* pol I cocrystal, whereas ACN-LAG led to the 1:1 *RS/L* pol II (samples 1, 5, 9, 13 in Tables S6.1 and S7.1†). When the initial ratio of the components in laboratory EtOH- and ISPN-LAG was taken so that pro was present in an excess amount (1:2 or 2:3 nprx:pro ratio), the final powders, besides the 1:1 *RS/L* pol I, also contained the 1:2 *RS/L* phase (see samples 6, 8, 10, and 12 in Tables S6.1 and S7.1†). Varying the ratio between the components in MeOH- and ACN-LAG reactions, led to the 1:1 *RS/L* pol I and 1:1 *RS/L* pol II, respectively, and some of the initial material as impurity (see samples 2–4 and 14–16 in Tables S6.1 and S7.1†), except the 2:3 ACN-LAG reaction (sample 16 in Table S7.1†) where the presence of the 1:2 *RS/L* as a co-product was detected as well. Hence, the 1:1 *RS/L* pol II was detected only in the ACN-LAG reactions; 1:2 *RS/L* was observed mainly in the ISPN- and EtOH-LAG, often as a co-product of the 1:1 *RS/L* pol I.

The results of the *in situ* ball-milling experiments for this system (Fig. 7) differed from our laboratory LAG reactions: grinding with all four tested solvents led to the formation of the 1:1 *RS/L* pol I phase, showing no traces of the 1:1 *RS/L* pol II even in ACN-LAG, which in the laboratory experiments always resulted in the 1:1 *RS/L* pol II (samples 9–12 in Table S3.1†). Distinguishing between the two 1:1 polymorphs was complicated due to their close diffraction patterns. In order to be sure that we do not have the pol II in the final powders we remeasured the PXRD data for those samples at the laboratory diffractometer (Fig. S3.1.11†): all the four samples after 1:1 MeOH-, EtOH-, ISPN- and ACN-LAG reactions contained only the 1:1 *RS/L* pol I.

MeOH- and ISPN-LAG *in situ* ball-milling showed the formation of only the 1:1 *RS/L* pol I (Fig. 7a and d, S3.1.5 and S3.1.7†). These two reactions differed only by the reaction rate, which was slower in the case of ISPN-LAG (in the latter, the initial reactants disappeared only by the 6th min of reaction against before the 1st min in the case of MeOH-LAG).

In situ EtOH-LAG showed the emergence and then disappearance of the 1:2 *RS/L* phase at the early stage of reaction (Fig. 7b and S3.1.6†). Moreover, ACN-LAG revealed the emergence of another unknown phase (unknown phase IV) whose diffraction pattern did not correspond to any previously identified phases (Fig. 7c and S3.1.8†). Unfortunately, due to the poor quality of the data, reliable determination of this phase was impossible.

Hence, *in situ* ball-milling data showed that the reaction pathway differs depending on the solvent used. Since the results were inconsistent with the preliminary laboratory grinding experiments, we conclude that the reaction is highly sensitive to the milling conditions, which renders a direct comparison of the laboratory grinding and *in situ* ball-milling unreliable in this case.

Additional *in situ* ball-milling experiments were performed for the 1:2 MeOH- and ISPN-LAG reactions (samples 13 and 14 in Table S3.1†) in order to monitor the formation of the

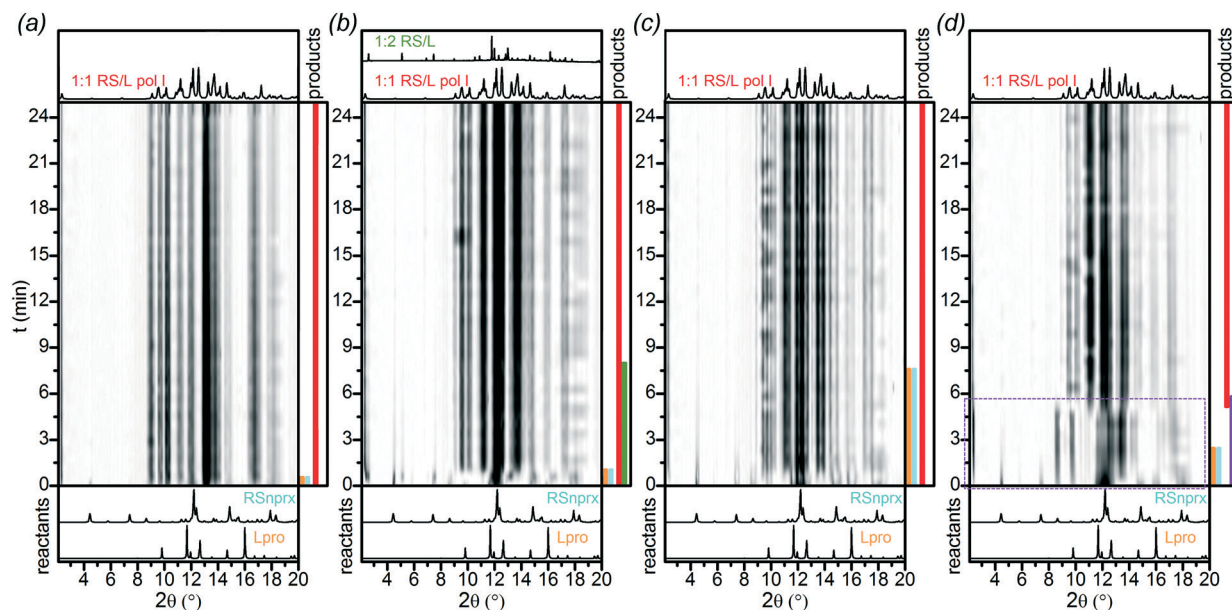


Fig. 7 *In situ* ball-milling X-ray diffraction patterns for the RS/L system, $\lambda = 1 \text{ \AA}$, 1:1 ratio: (a) MeOH-, (b) EtOH-, (c) ISPN-, and (d) ACN-LAG. Colored bands on the right side of each image represent the presence of phases and are shown only for illustrative purposes; the area outlined by purple in (d) shows the presence of the unknown phase IV.

1:2 RS/L. Unfortunately, the MeOH-LAG data had a very poor quality and we were able to collect only the PXRD pattern of the final powder, which showed no presence of 1:2 RS/L, only 1:1 RS/L pol I and the initial compounds as impurities (Fig. S3.1.10†). The ISPN-LAG, on the other hand, clearly showed the formation of the 1:2 RS/L, that started emerging later (around the 4th min of reaction) than the 1:1 RS/L pol I (which was observed already after 1 min). The final powder contained both the 1:1 RS/L pol I and 1:2 RS/L (Fig. S3.1.9 and S3.1.18†). These two results were consistent with our laboratory data, in which the formation of the 1:2 RS/L phase was observed only in ISPN-LAG (sample 10 in Tables S6.1 and S7.1†), whereas 1:2 MeOH-LAG showed no traces of this phase, but only the 1:1 RS/L pol I (sample 2 in Tables S6.1 and S7.1†).

Variable-temperature *in situ* PXRD helped in structure determination of the 1:2 RS/L phase, providing access to its pure diffraction pattern (Fig. 8). The 1:2 RS/L phase was

always obtained in a mixture with other phases, and thus indexing and structure determination were tricky at ambient conditions. The variable-temperature experiment showed that the 1:2 RS/L phase is more stable upon heating and thus disappears later than the 1:1 RS/L pol I: the sample in the temperature range from 127 to 144 °C contained almost pure 1:2 RS/L (the amount of L-pro was negligible).

Water-LAG in this system yielded the already known 1:1 RS/L/H₂O cocrystal hydrate (CCDC REF BEXGUI,²⁸ samples 17–19 in Tables S6.1 and S7.1†). Using variable-temperature *in situ* PXRD for this sample, we found another polymorph, 1:1 RS/L pol III (Fig. 9), which was not observed in any other LAG experiments. This new pol III emerged upon heating when water evaporated from the initial hydrated sample; upon further heating pol III disappeared followed by the appearance of the 1:1 RS/L pol I. From the structural point of view, the 1:1 RS/L/H₂O and 1:1 RS/L pol III exhibit

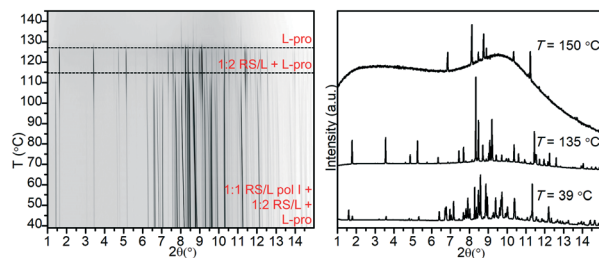


Fig. 8 PXRD patterns measured *in situ* upon heating (from 39 to 163 °C, 2 °C min⁻¹) for the RS/L sample prepared by 1:2 ISPN-LAG (sample 10 in Table S7.1†), $\lambda = 0.708 \text{ \AA}$: (left) film representation; (right) XRD patterns at selected temperatures: $T = 39 \text{ °C}$ – 1:1 RS/L pol I, 1:2 RS/L, and L-pro; $T = 135 \text{ °C}$ – 1:2 RS/L and L-pro; and $T = 150 \text{ °C}$ – L-pro.

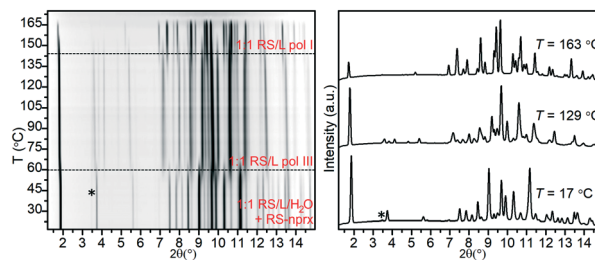


Fig. 9 PXRD patterns measured *in situ* upon heating (from 17 to 175 °C, 2 °C min⁻¹) for the RS/L sample prepared by 1:1 water-LAG (sample 17 in Table S7.1†), $\lambda = 0.78487 \text{ \AA}$: (left) film representation; (right) XRD patterns at selected temperatures: $T = 17 \text{ °C}$ – 1:1 RS/L/H₂O, RS-nprx (marked as *); $T = 129 \text{ °C}$ – 1:1 RS/L pol III; and $T = 163 \text{ °C}$ – 1:1 RS/L pol I.

completely different types of packing: the former being of type I and the latter of type II (see Structural analysis section for the description of the packing types and Fig. S10.2† for the comparison of this particular case). Such structural differences show that water evaporation from the hydrated 1:1 *RS*/*L*/*H*₂*O* cocrystal induces a rearrangement of the entire structure, leading to a cocrystal with a completely different structural type than other cocrystal forms from this system (which all exhibit the type I packing).

In conclusion, the *RS*/*L* system exhibits cocrystals with various stoichiometries (1:1 and 1:2), including a hydrate and a solvate. *In situ* ball-milling showed that LAG reactions are highly sensitive to the solvent used as well as the experimental setup and also revealed that even if the final product is the same, the reaction pathway is solvent-dependent. Variable-temperature *in situ* PXRD data helped in determining the structure of the 1:2 *RS*/*L* phase and gave access to the 1:1 *RS*/*L* pol III that was not observed under any other conditions.

RS-Naproxen/*DL*-proline

For the *RS*/*DL* system we found two polymorphs with the 1:1 stoichiometry – 1:1 *RS*/*DL* pol I and 1:1 *RS*/*DL* pol II – and one 1:1 *RS*/*DL*/*H*₂*O* cocrystal hydrate (the structure of the cocrystal hydrate was previously reported, CCDC BEYTUW²⁸). We also established the formation of the (1:2 *R*/*DL* + 1:2 *S*/*DL*) conglomerate under certain conditions (Scheme 1).

In general, the laboratory LAG experiments mainly yielded the 1:1 *RS*/*DL* pol I cocrystal (Table S8.1†). The formation of the conglomerate was observed when the initial *RS*-npx and *DL*-pro were ground in 1:2 or 2:3 ratio, often as a coproduct of the 1:1 *RS*/*DL* pol I phase (samples 2, 4, 6, 8, 10, 12, 14, 16 in Table S8.1†); the presence of the (1:2 *R*/*DL* + 1:2 *S*/*DL*) conglomerate was also detected in the 1:1 *ISPN*-LAG (sample 9 in Table S8.1†). The fact that we dealt exactly with the conglomerate and not any other combination of phases was confirmed by variable-temperature *in situ* PXRD. The sample obtained by 1:2 MeOH-LAG was heated in a capillary. Initially, the sample contained a mixture of different phases: 1:1 *RS*/*DL* pol I, (1:2 *R*/*DL* + 1:2 *S*/*DL*) conglomerate, and *DL*-pro monohydrate. Upon heating, first *DL*-pro monohydrate transformed into unhydrated *DL*-pro, which upon further heating disappeared along with the 1:1 *RS*/*DL* pol I phase, leaving only the pure conglomerate (Fig. 10). The *in situ* variable-temperature PXRD data are in agreement with the DSC, which show that the melting point of the 1:1 *RS*/*DL* pol I phase is lower than the eutectic point of the conglomerate (152(1) against 168(1) °C, respectively) and thus, the former should melt before the latter.

In situ 1:1 ball-milling, neither in the presence of MeOH nor *ISPN*, showed conglomerate formation (samples 15 and 16 in Table S3.1†). *ISPN*-LAG led directly to the 1:1 *RS*/*DL* pol I phase (Fig. 11b). MeOH-LAG revealed 1:1 *RS*/*DL*/*H*₂*O* emerging at the beginning of the reaction and then disappearing shortly (Fig. 11a and S3.1.12†).

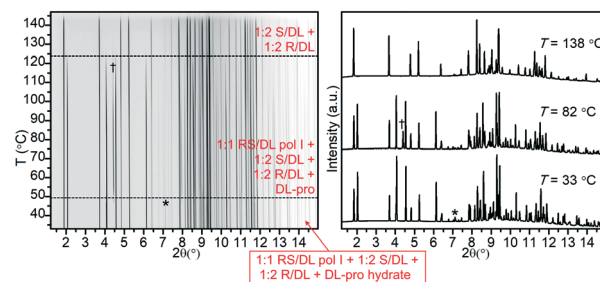


Fig. 10 PXRD patterns measured *in situ* upon heating (from 33 to 147 °C, 2 °C min⁻¹) for the *RS*/*DL* sample prepared by 1:2 MeOH-LAG (sample 2 in Table S8.1†), $\lambda = 0.708$ Å: (left) film representation; (right) XRD patterns at selected temperatures: *T* = 33 °C – 1:1 *RS*/*DL* pol I, (1:2 *R*/*DL* + 1:2 *S*/*DL*) conglomerate, and *DL*-pro monohydrate; *T* = 82 °C – 1:1 *RS*/*DL* pol I, (1:2 *R*/*DL* + 1:2 *S*/*DL*) conglomerate, and *DL*-pro; and *T* = 138 °C – (1:2 *R*/*DL* + 1:2 *S*/*DL*) conglomerate.

On the other hand, when the ratio was changed to 1:2, *in situ* ball-milling in the presence of both MeOH and *ISPN* showed the formation of the conglomerate (samples 17 and 18 in Table S3.1†). In 1:2 MeOH-LAG, the final powder contained both the conglomerate and the 1:1 *RS*/*DL* pol I: the conglomerate started to emerge first and the 1:1 phase followed (Fig. 11c and S3.1.13†); moreover, we detected again the formation of the 1:1 *RS*/*DL*/*H*₂*O* within the 1st min of reaction, which later disappeared. In 1:2 *ISPN*-LAG, the final sample contained only the conglomerate, whose emergence was preceded by the 1:1 *RS*/*DL* pol I phase, which disappeared already after 8 min of grinding (Fig. 11d and S3.1.14†). These experiments show that the conglomerate and the 1:1 *RS*/*DL* pol I phase kinetically compete with each other and the result significantly depends on the reaction conditions.

Water-assisted laboratory grinding yielded the 1:1 *RS*/*DL*/*H*₂*O* cocrystal hydrate (samples 17–19 Table S8.1†), which upon heating transformed into the 1:1 *RS*/*DL* pol II phase when water evaporated from the sample. Upon further heating the 1:1 *RS*/*DL* pol II simply melted without any other phases emerging (Fig. 12). The 1:1 *RS*/*DL* pol II cocrystal was not detected upon any other tested conditions. Comparing 1:1 *RS*/*DL*/*H*₂*O* and 1:1 *RS*/*DL* pol II showed that the hydrated structure undergoes a complete structural rearrangement upon heating, with the final unhydrated structure being of the type II packing, unlike the initial hydrated form and the 1:1 *RS*/*DL* pol I cocrystal, both belonging to the type I packing (see Structural analysis section for the description of the packing types and Fig. S10.3† for the comparison of this particular case).

Hence, the most interesting finding for the *RS*/*DL* system is the formation of the (1:2 *R*/*DL* + 1:2 *S*/*DL*) conglomerate under certain conditions. *In situ* variable-temperature PXRD data were essential to prove this fact as they provided access to the diffraction pattern of the pure conglomerate. Another *in situ* variable-temperature PXRD experiment allowed structure solution of the 1:1 *RS*/*DL* pol II, as this phase was

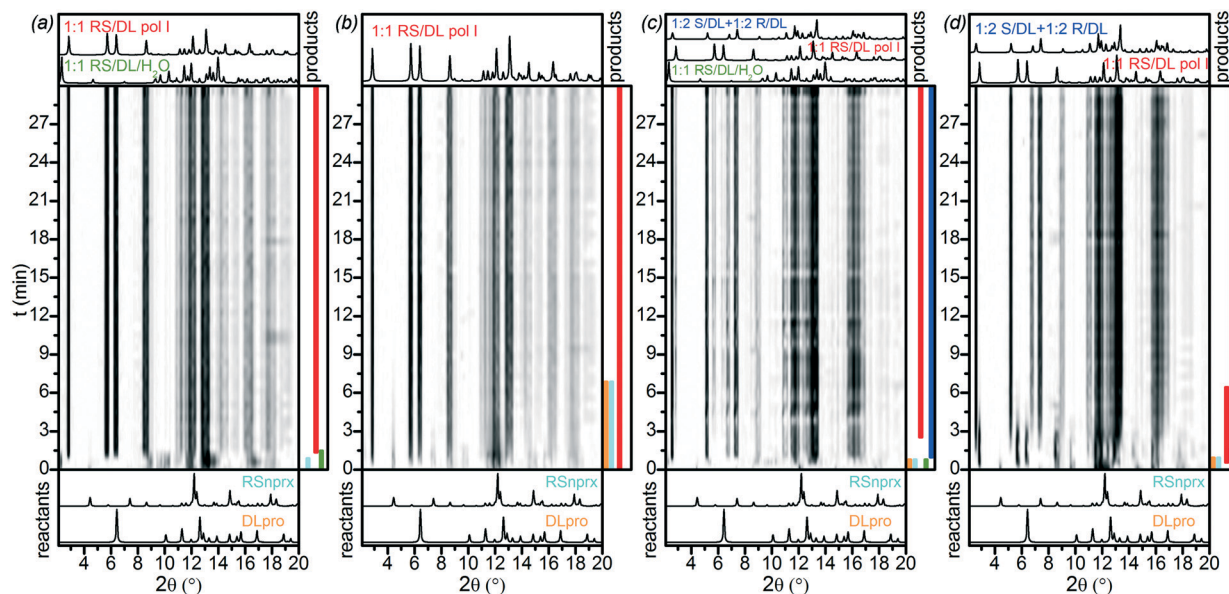


Fig. 11 *In situ* ball-milling X-ray diffraction patterns for the RS/DL system, $\lambda = 1 \text{ \AA}$: (a) 1:1 MeOH-, (b) 1:1 ISPN-, (c) 1:2 MeOH- and (d) 1:2 ISPN-LAG. Colored bands on the right side of each image represent the presence of phases and are shown only for illustrative purposes.

observed only upon heating and not under any other tested conditions.

Structural analysis

In our previously published work,⁴¹ we studied the flurbiprofen/proline system, which similarly to naproxen/proline showed a great variety of cocrystal phases. Below, we will analyse the structural features of nprx/pro cocrystals and compare them to those seen in the flurbiprofen/proline system.

Unlike flurbiprofen/proline cocrystals, for which we distinguished 4 packing types,⁴¹ nprx/pro cocrystals exhibit less variability – only two types of packing were found (Scheme 2): 1) type I – sandwich-like type, in which pro chains form a center layer with nprx attached from both sides; 2) type – layer type, in which one can distinguish alternating layers of pro and nprx, with nprx arranged in a zip-like manner. Moreover, type I can be divided into two subtypes, a and b, which

differ from one another by the arrangement of nprx molecules: in the Ia subtype, nprx packs in a herringbone manner and in the Ib subtype nprx molecules are parallel. Schematic representation of these packing types and illustrating examples are given in Fig. 13.

Pro is zwitterionic in all the structures and forms head-to-tail chains through charge-assisted N-H $\cdots$ O hydrogen bonds, which are typical of crystalline pro and other amino acids.^{25–27} Nprx attaches to pro *via* O-H $\cdots$ O hydrogen bonds between the carboxyl group and the carboxylate, respectively.

The fact that nprx/pro cocrystals vary less in their structural features than flurbiprofen/proline cocrystals may be related to the structural peculiarities of nprx and flurbiprofen themselves. Pure flurbiprofen exhibits polymorphism (CCDC FLUBIP⁶² (*P* $\bar{1}$) and FLUBIP02 (ref. 63) (*P*₂₁/*n*)) and we found that the packing in cocrystal structures resembled that of pure flurbiprofen, whereas in the case of nprx, no polymorphism has been reported so far. Both racemic and

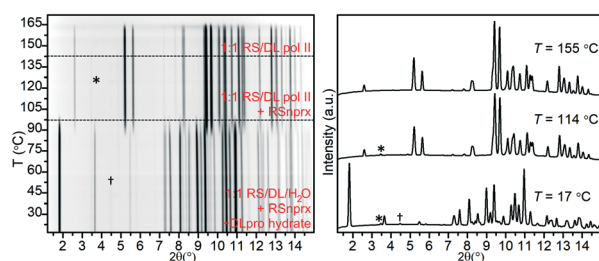
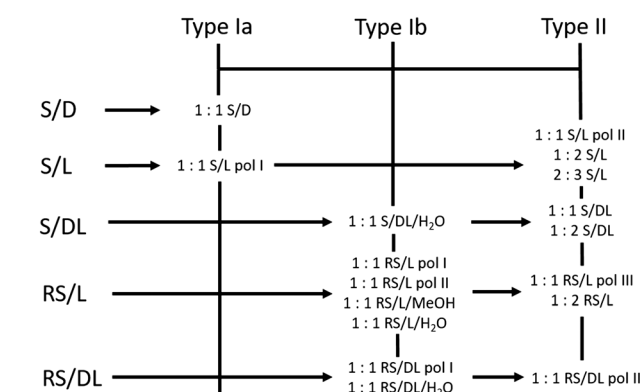


Fig. 12 PXRD patterns measured *in situ* upon heating (from 17 to 170 °C, 2 °C min⁻¹) for the RS/DL sample prepared by 1:1 water-LAG (sample 17 in Table S8.1†), $\lambda = 0.78487 \text{ \AA}$: (left) film representation; (right) XRD patterns at selected temperatures: $T = 17 \text{ °C}$ – 1:1 RS/DL/H₂O, RS-nprx (marked as *), and DL-pro monohydrate (marked as +); $T = 114 \text{ °C}$ – 1:1 RS/DL pol II and RS-nprx; and $T = 155 \text{ °C}$ – 1:1 RS/DL pol II.



Scheme 2 Structural types distinguished for the nprx/pro cocrystals.

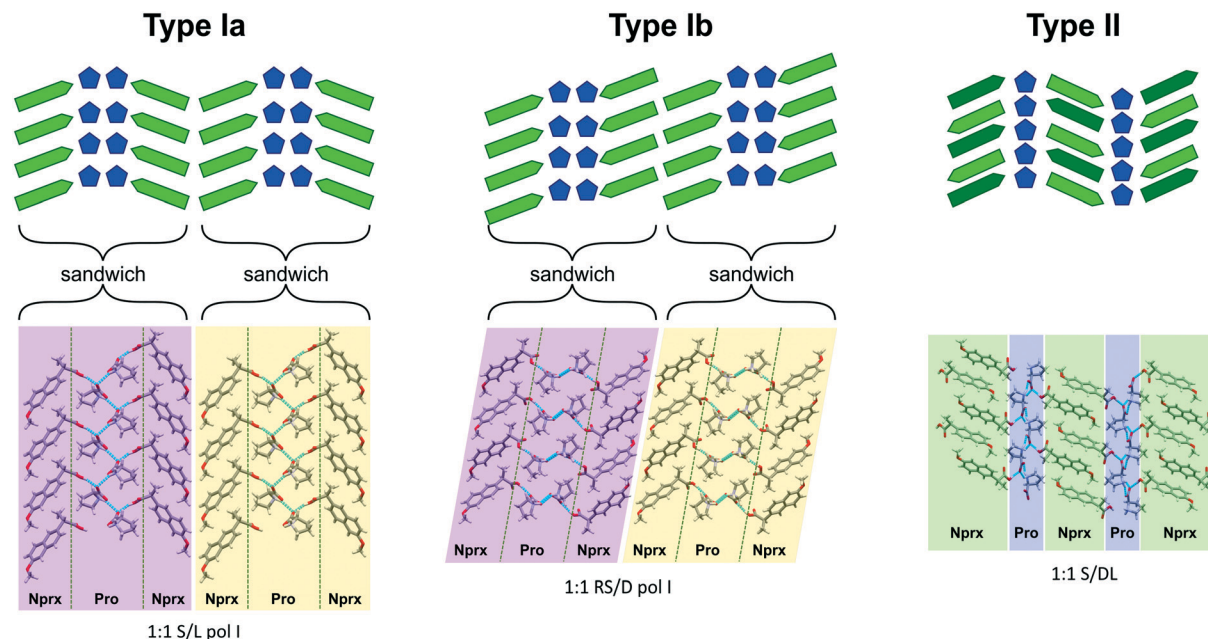


Fig. 13 Schematic representation of the structural types distinguished for the nprx/pro system. Blue pentagons and green pointed (the COOH group orientation) rectangles represent pro and nprx molecules, respectively. Type I is the sandwich-like type, separate sandwiches are colored pink and yellow in the structure examples; type II is the layer type: alternating nprx and pro layers are colored green and blue, respectively, in the structure example.

enantiopure nprx structures exhibit a similar general packing motif: nprx dimers are arranged in a stacking manner to enable efficient π - π overlap between the benzene rings. *S*-nprx/ethanol solvate, which we obtained from ethanol solution, also exhibited the same general packing as non-solvated nprx, with ethanol molecules breaking the nprx dimers and forming infinite ABABAB (A – nprx, B – ethanol) chains (see Fig. S10.1†). Since ethanol was essential to keep the structure together, the latter turned out to be very unstable if exposed to air. Nprx is more rigid as compared with flurbiprofen, which may cause nprx to be less prone to polymorphism and also result in fewer variants of packing in cocrystals.

Previously reported cocrystals of nprx and amino acids¹⁷ also demonstrated the same types of packing: nprx/alanine (CCDC RODSEK and RODSIO¹⁷) and nprx/tryptophan (CCDC RODSOU¹⁷) had the sandwich-like type (I) and nprx/tyrosine (CCDC RODSUA¹⁷) exhibited the layer type (II) packing. Hence, we suspect that since nprx prefers a stacking manner of molecular arrangement, the weak interactions between its bulky molecules are responsible for the overall packing of nprx/amino acid cocrystals.

At the same time, we believe that amino acid chains, with strong charge-assisted hydrogen bonds between molecules, should provide the structure with additional stability and a higher melting point in comparison with the structure of the initial API as the percentage of strong interactions in the structure increases. For instance, the melting point of flurbiprofen/proline cocrystals was a minimum 30 °C higher in comparison with that of pure flurbiprofen (T_m (racemic flurbiprofen) = 115 °C, T_m (enantiopure flurbiprofen) = 109

°C).⁴¹ The melting points of some of the nprx/pro cocrystals were also found to be higher than that of pure nprx (see Table 1). The highest melting points were observed for the cocrystals with the 1:2 and 2:3 stoichiometries, which can be explained by the fact that these cocrystals exhibit a higher percentage of charge-assisted hydrogen bonds as compared with the 1:1 phases due to an extra pro molecule in the asymmetric unit. The 1:1 phases, on the other hand, were found to have melting points very close to that of pure nprx, which highlights the fact that the interactions between nprx molecules themselves are substantial and provide efficient bonding even without strong charge-assisted hydrogen bonds.

Another peculiar finding about nprx/pro cocrystals is the cocrystals of the 1:2 and 2:3 stoichiometries, in which nprx is connected only to one out of the two pro molecules in the asymmetric unit of the 1:2 cocrystals, and to two out of the

Table 1 Determined melting points of the initial API and its cocrystals with pro

Compound	Melting point, °C
<i>S</i> -nprx	155(1)
<i>RS</i> -nprx	153(1)
2:3 S/L	181(1)
1:1 S/D	155(1)
1:1 RS/L pol I	162(1)
1:1 RS/L pol II	159(1)
1:2 RS/L	171(1)
1:1 RS/DL pol I	152(1)
1:2 S/DL	182(1)
(1:2 S/DL + 1:2 R/DL)	168(1) – eutectic point

three molecules in the cocrystals of the 2:3 stoichiometry. In Scheme 3 we present four possibilities of the interactions between nprx and pro in an asymmetric unit. All the five combinations of racemic and/or enantiopure nprx and pro yielded at least one 1:1 stoichiometry cocrystal (case (a) in Scheme 3). Cocrystals of this type were seen in abundance in both the nprx/pro and flurbiprofen/pro systems. A cocrystal of the 1:2 stoichiometry (case (b) in Scheme 3) was also observed in the flurbiprofen/pro system (CCDC VEVLAM⁴¹) and it turns out that its structure is very similar to that of the 1:2 nprx/pro cocrystals: two perpendicularly going infinite pro chains form a pro layer, in which nprx is connected only to one of those chains (see Fig. S10.4†). In the 2:3 *S/L* cocrystal (case (c) in Scheme 3) the pro layer is composed of clusters situated one on top of the other and made up of three interconnected pro chains; nprx is attached to only two chains from the cluster (see Fig. S10.5†). The last type of interactions, case (d), in which one pro molecule attaches two APIs *via* two O–H...O hydrogen bonds formed by its carboxylate group with two carboxyl groups of an API, resulting in the cocrystals with the 2:1 (API:pro) stoichiometry, was not observed in the nprx/pro system, even though it was very common among the flurbiprofen/pro cocrystals.

Cocrystals with the aforementioned 1:2 and 2:3 stoichiometries are less common. Their existence shows that in order to obtain a cocrystal of the desired stoichiometry it is not enough to simply choose the compounds with potential binding sites, but it is also important to consider the possibility of other potentially stronger interactions that might occur between the molecules of one kind, like in this case,

when instead of binding a second nprx to the available oxygen of the carboxylate, pro preferred to bind another symmetrically nonequivalent pro molecule and form a 1:2 or 2:3 (nprx:pro) cocrystal.

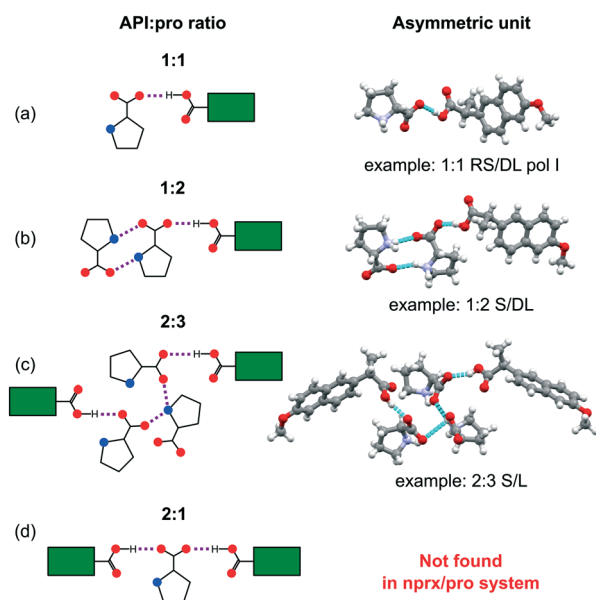
Conclusions

The nprx/pro system exhibits a wide variety of cocrystal forms, 17 in total, whose structures were determined, plus four unknown phases whose structures were impossible to find. Similarly to the previously reported case of flurbiprofen/proline cocrystals,⁴¹ we believe that the complexity of the system can be partly associated with how well the three-dimensional structures of the initial compounds “suit” one another to form an efficient packing pattern.

The system might have a higher possibility of efficient packing in the case of racemic initial compounds in comparison with the enantiopure ones because having both enantiomers in the structure may increase the chances of “suitability”. At the same time, some of the chiral combinations may form stronger connections than the others. For instance, the *S/D* system showed only one cocrystal, whereas the *S/L* system yielded a minimum of four cocrystal phases. Probably, in the case of the *S/D* combination, the interactions between coformers are so efficient that they do not leave any chance for other polymorphs or stoichiometrically diverse cocrystals to emerge under the studied conditions as the former is both thermodynamically and kinetically favored, whereas in the *S/L* system, the initial coformers might fit one another to a lesser degree and, thus, many competitive phases (probably of approximately the same energies) may emerge. This conclusion is speculative on our part, and we suggest that a careful calculation of the relative energies of the obtained cocrystals can shine more light on this chirality-related aspect of cocrystallization.

The melting points that we were able to determine for several nprx/pro cocrystals turned out to be higher than that of pure nprx, which is due to pro's ability to form charge-assisted hydrogen bonded chains owing to which the percentage of strong interactions in the structure increases in comparison with the pure API. As all amino acids in the crystalline state are zwitterionic and tend to form such chains,^{25–27} which are also frequently observed in their cocrystals,^{17,28,64} this structural feature of amino acid cocrystals can be especially useful when handling unstable APIs: one of the reported examples is the improvement of the stability of highly hygroscopic lactic acid *via* its cocrystallization with tryptophan.²⁹

In situ ball-milling monitored by synchrotron X-ray diffraction has already proved vital for studying solid-state reactions.^{58,65–68} Knowing at which moment to stop the reaction in order to obtain a desired product (for instance, one of the intermediates) can provide a better control of the reaction outcome. In the current study, we used this method to verify the possibility of intermediate phases in our LAG reactions. We detected several intermediate phases emerging in the



Scheme 3 Four possibilities of interactions between profen (API) and proline molecules in an asymmetric unit of their cocrystals. (a)–(c) cases are observed in the nprx/pro system; (d) case, typical of the flurbiprofen/pro system, was not detected for nprx/pro. Red circles represent oxygen atoms, blue circles represent nitrogen, green rectangles with attached –COOH group represent a profen molecule (API).

course of the reaction. We found that the reaction pathway can be solvent dependent, as for instance, in the case of the *S/L* system, when in the beginning of the reaction we observed different phases depending on the solvent used, even though the final product was the same. How a mechanochemical reaction proceeds in the presence of various solvents may depend on the solubility of the initial compounds: when both compounds have relatively good solubilities the reaction proceeds faster (as we saw in the cases of methanol or ethanol), but the reaction may slow down when only one of the coformers is soluble (in isopropanol and acetonitrile, only *nprx* is soluble whereas *pro* exhibits very poor solubility). According to one of the mechanisms, adding liquid facilitates the diffusion of molecules from the reactant to the product phase *via* an intermediate solution phase; moreover, the rate of diffusion is inversely proportional to the particle size and thus increases during grinding.^{69,70} The nature of the solvent may also affect the surface properties of the reactants, for instance, by rendering the reacting powder sticky, which prevents good mixing.⁷¹ Altogether, these factors, among others such as the intensity of mechanochemical treatment, the material the jar is made of, the crystallite size of the initial materials (smaller crystallites offer a larger reactive surface area), the amount of liquid in proportion to the powder material, can be responsible for the reaction rate, reaction outcome or emerging intermediates.

Synchrotron X-ray diffraction data turned out to be indispensable when dealing with complicated product mixtures. Similarly to our work on flurbiprofen/proline cocrystals, synchrotron X-ray diffraction data collected *in situ* upon heating allowed direct monitoring of phase changes and gave access to the pure PXRD patterns of certain phases. They allowed structure solution of the 1:2 *RS/L* cocrystal, otherwise observed only in mixtures, helped us to confirm the conglomerate formation in the *RS/DL* system, and revealed the 1:1 *RS/DL* pol II and 1:1 *RS/L* pol II phases that emerged when the hydrated forms of the corresponding cocrystals were heated and water evaporated.

The results of this work should be of great interest to the pharmaceutical industry as they deal with various chirality aspects of cocrystallization of APIs, provide insight into various structural features of cocrystals with zwitterionic coformers *via* the example of *pro*, and demonstrate how clever combining various state-of-the-art characterization methods helps in not only identifying existing phases but also providing means for a better control of solid-state reactions.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The authors thank the Universite catholique de Louvain and the FNRS (FRIA scholarship allocated to N. Tumanova) for financial support. We thank the ESRF, PSI, BESSY II, and

SOLEIL for the beam time allocation. We thank the PC² platform at the University of Namur for single-crystal and powder X-ray diffraction analysis. Natalia Tumanova is grateful to Dr. F. Emmerling's group for a warm welcome during her STSM funded by COST Crystallize Action (CM1402). The authors highly appreciate advice from Dr. Radovan Cerny concerning the structure solution from PXRD. We thank Lisa Baulard for her help in preparing solutions for single-crystal growth, Pascal Van Velthem for helping us to treat DSC data, and Jean-François Statsyns for measuring the TGA data.

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