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**HIGHLIGHT**

# Some thoughts about the single crystal growth of small molecules†

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This highlight critically compares various techniques to grow single crystals when only a few milligrams are available of the compound of interest. The authors describe vapour diffusion, evaporation, cooling, and layering techniques, as well as crystallisation in gels. A table of successfully applied solvent/antisolvent combinations for initial screening is given. Additionally, a comprehensive table of 107 solvents with their boiling points, densities and dielectric constants helps to optimise the crystal growth.

## Introduction

Formation of suitable single crystals undoubtedly is the most decisive step of a successful single crystal X-ray structure determination. Quite often, only an X-ray analysis will definitively reveal the composition and three-dimensional arrangement of an unknown compound. The use of area detectors often allows finishing a complete X-ray structure analysis within a few hours, making it a fast analytical method. However, this powerful technique fully relies upon the ability to grow single crystals of sufficient quality and size. Although crystallisation is an important purification technique which is taught and widely used in every chemistry undergraduate curriculum, different techniques may have to be employed for the growth of diffraction-quality single crystals.

One of the useful descriptions about growing single crystals is that of Jones.[1] Kroon and co-workers have summarised the different techniques for growing crystals of organic molecules, though their methods can be extended to many small molecules.[2] Hulliger has described in great detail techniques that are suitable for various types of compounds.[3] On the internet, many pages are dedicated to the description of how to grow single

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crystals.[4,5] The crystallisation of active pharmaceutical ingredients has been reviewed.[6] It needs to be noted that crystallisation is not only the most important purification method for the pharmaceutical industry. Crystal polymorphs also play an extremely crucial role in terms of processing, bioavailability, stability, regulatory affairs, and intellectual property protection.[7–9] Not surprisingly, several publications have studied the influence of solvents on the crystallisation of polymorphs.[10,11] Of course, the use of different solvents often results in solvate crystals as shown for organic crystals,[12] metal complexes,[13] or pharmaceuticals.[14–16] Since the number of possible solvents is very large, solvents were grouped after statistical analysis of selected solvent parameters.[17–19] However, the knowledge gained from these investigations was not used to tackle an open crystallisation challenge.

Furthermore, different polymers were used as heteronuclei to find new polymorphs.[20] Finally, it is clear that the crystallisation behaviour is dominated by the intrinsic properties of the very compounds.[21,22] There will always be substances that are easier to crystallise than others. Nevertheless, it has been our experience in the last fifteen years that beginners are often lacking the knowledge of how to setup crystallisation trials. Secondly, even experienced researchers are grateful for hints of how to optimise crystallisation setups that hitherto have failed to produce suitable single crystals. For the following discussions, we will assume that the compound of interest is available in small amounts of not more than 50 milligrams only. This quite common situation has severe consequences: a quantitative determination of the solubility in common solvents is not possible. Furthermore, some crystallisation techniques cannot be used, because they require more material and they do not allow the recovery of the compound of interest.

This paper mainly deals with the crystallisation of small molecules and has a two-fold aim: for beginners in the field of single crystal growth, we want to describe and critically compare the most commonly used basic techniques for the growth of single crystals: (a) vapour diffusion (including solvent evaporation) and (b) solvent layering. We briefly also discuss the unconventional technique of crystal growth in gels. At the same time, we want to give the more advanced researchers some ideas, in case their standard methods fail to work. As a starting point, we present a few solvent combinations that proved to be very useful in our hands for vapour diffusion. In addition, we will show that a careful screening of the solvents, including some uncommon ones, increases the chances of obtaining single crystals. These two instances are exemplified by the case studies of diethyl-4,4′-dipyridin-4-yl-2,2′-bipyrrole-3,3′-dicarboxylate (**1**) and 3-carbethoxyquinoline (**2**). Throughout this highlight, we will share our experience about various aspects of crystallisation that we have accumulated over the last fifteen years.

## Results and discussion

In our hands, most compounds that are amenable to thermal recrystallisation can also be crystallised to yield single crystals. Furthermore, we experienced that thermal recrystallisation and cooling of solutions in a refrigerator or freezer are inferior to isothermal methods and only rarely give rise to suitable single crystals. When trying to use such methods, the corresponding mother liquids should be cooled down as slowly as possible. We prefer instead, as discussed below, the techniques of vapour diffusion and layering experiments backed up by an appropriate solvent choice. As a general remark, the temperature and its stability during the crystallisation experiment can be of outmost importance. The stability as well as the possible variance of temperature between day and night can be helpful for the crystal growth. However, we have very rarely found it necessary to use a thermostatically controlled cabinet.

### 1. Crystallisation by vapour diffusion (and solvent evaporation)

The experimental setup consists of an inner container with the compound of interest to be crystallised (from now on "solute") in a solvent and an outer container with an antisolvent.[23] Both containers share a common gas phase (Fig. 1, left). Normally, we use about 4 mg of solute. More material is often inappropriate and would only increase the chances for crystal intergrowth within the employed container size. About 0.5 ml of solvent is placed in the inner tube and 2.5 ml of antisolvent in the outer container. The exact amount of solvent depends upon the solubility of the solute. If necessary, the solution of the solute is

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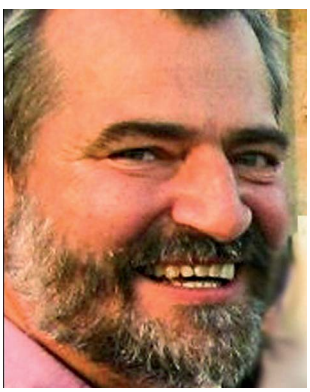

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**Fig. 1** Setup for vapour diffusion (left) and layering (right) exemplified with aqueous vitamin $B_{12}$ solutions (acetone as an antisolvent). In the case of the vapour diffusion, the antisolvent acetone must be refilled several times for crystal growth. The image has been edited in order to make the transparent layer of the antisolvent within the NMR tube more visible.

filtered. Then, the outer container is closed and the two liquids start to equilibrate *via* vapour diffusion, which will hopefully result in single crystal formation. We only use tablet tubes as the inner container, since they have no neck which simplifies the removal of the crystals.

Furthermore, the tablet tubes allow a complete examination of the vial with the help of a microscope. Besides the missing neck, the relatively flat bottom of tablet tubes also facilitates an optical inspection in vertical orientation. Some crystallographers recommend covering the inner tube with, *e.g.* a punctured parafilm or aluminium foil. They have observed that the slowed down diffusion can have beneficial effects.[24] We normally do not cover the inner container as this complicates inspection of the inner container with the help of a microscope. An appropriate choice of solvent and antisolvent (see below) also ensures that the vapour equilibration takes place during several days, which seems to be an ideal time period.

In contrast to some published procedures,[5] we generally choose an antisolvent having a higher boiling point than the solvent. We do this for the following reasons:[25] if the solvent is more volatile than the antisolvent, the solvent will evaporate into the antisolvent, thus increasing the concentration of the solute. This effect is additionally superimposed by an increasing amount of antisolvent diffusing into the solvent and thus reducing the solubility of the solute. Both effects are synergistic. In contrast, if the antisolvent is more volatile (like diethyl ether), it will rapidly diffuse into the solvent. Therefore, the overall concentration will be reduced, even if the solubility is lowered. According to our observations, this is rarely accompanied by any phase change or even crystal growth. Consistent with these considerations, quite often the solute only starts to form a solid (and ideally crystalline) phase after a significant portion of the solvent has evaporated.

For initial vapour diffusion experiments, we found the solvent combinations shown in Table 1 to be a good starting point. Ideal solvents (or antisolvents) should moderately solubilise (or desolubilise) the solute respectively. The transparent containers allow a continuous inspection of the crystallisation experiment without the need to touch or move the setup. After removal of the screw cap, inspection of the crystallisation setup with the help of a through-light microscope can be done. If after two weeks, with not too high boiling solvents, no phase change has occurred, the screw cap of the outer container is slightly unscrewed. The system is now open and a slow evaporation of the solvents/antisolvents is achieved. At this point, the vapour diffusion experiment turns into a mixed solvent evaporation experiment. Obviously there is also a strong solvent dependence on the outcome of an evaporation crystallisation. We will later discuss this phenomenon and how to optimize this process.

As part of the procedure, the liquid in the outer container must be removed, as soon as crystals in the inner tube are detected. By this, a subsequent cocrystallisation of possibly present impurities is avoided.

If the solute turns into an oil or precipitation occurs, the experiment should be stopped and repeated with other solvents of different compound classes.[23] From our experience, single crystals are very rarely obtained after oil or precipitate formation. We have previously shown tetrahydropyran to be a solvent that is useful for crystallisation.[27] The 107 solvents listed in Table 2 should be an inspiration as to which other solvents could be tested if the initial crystallisation trials were not successful. Since most crystallisations are accomplished at room temperature, the choice of solvents was restricted to those with a melting point below 20 °C and a boiling point above 30 °C. Only a very few solvents with a boiling point above 150 °C were selected. Additional considerations were given to the stability, toxicity and cost (for an acceptable purity) of the chosen solvents. Table 2 is especially useful if crystalline material was obtained that nevertheless was unsuitable for X-ray analysis. In such a case, slightly different solvents from the same compound class should be employed, *e.g.* 2-methyltetrahydrofuran instead of tetrahydrofuran. The solvents in the table are sorted according to their dielectric constants. From our experience, similar dielectric constants can serve as a rough guide to predict a similar solubility behaviour. Some exceptions from this empirical rule

**Table 1** Recommended solvents for vapour diffusion to explore initial crystallisation behaviour of small molecules

| Solvent | Antisolvent |
|---|---|
| Tetrahydrofuran | Cyclohexane |
| Methylformate | Cyclopentane or hexane (dries out) |
| Methylene chloride | Cyclopentane |
| Ethanol | Cyclohexane |
| Methanol | Hexane or tetrahydrofuran |
| Acetonitrile | Tetrahydropyran |
| Acetone | Chloroform |
| Water | Dioxane |

**Table 2** List of solvents suitable for either vapour diffusion or solvent layering, sorted by increasing dielectric constants. Data taken from ref. 26

| Sum formula | Name | Bp/°C | Density $\delta$/g ml$^{-1}$ | Dielectric constant $\varepsilon$ |
|---|---|---|---|---|
| $C_5H_{12}$ | Pentane | 36 | 0.626 | 1.84 |
| $C_6H_{24}$ | 2,2-Dimethylbutane | 58 | 0.662 | 1.87 |
| $C_6H_{14}$ | Hexane | 69 | 0.655 | 1.89 |
| $C_7H_{16}$ | Heptane | 99 | 0.684 | 1.92 |
| $C_8H_{18}$ | Octane | 126 | 0.699 | 1.95 |
| $C_5H_{10}$ | Cyclopentane | 49 | 0.746 | 1.97 |
| $C_6H_{12}$ | Methylcyclopentane | 72 | 0.749 | 1.99 |
| $C_7H_{14}$ | Methylcyclohexane | 101 | 0.769 | 2.02 |
| $C_6H_{12}$ | Cyclohexane | 81 | 0.774 | 2.02 |
| $C_6H_{12}$ | 1-Hexene | 63 | 0.673 | 2.08 |
| $C_4H_8O_2$ | 1,4-Dioxane | 102 | 1.034 | 2.22 |
| $C_6H_6$ | Benzene | 80 | 0.877 | 2.28 |
| $C_7H_8$ | Toluene | 111 | 0.867 | 2.38 |
| $C_8H_{10}$ | *o*-Xylene | 145 | 0.880 | 2.56 |
| $C_5H_6O$ | 2-Methylfuran | 65 | 0.913 | 2.76 |
| $C_5H_{10}O_3$ | Diethyl carbonate | 126 | 0.969 | 2.82 |
| $C_4H_4O$ | Furan | 32 | 0.951 | 2.88 |
| $C_8H_{18}O$ | Di-*n*-butyl ether | 140 | 0.768 | 3.08 |
| $C_2HCl_3$ | Trichloroethylene | 87 | 1.464 | 3.39 |
| $C_2H_4O_2$ | Diisopropyl ether | 69 | 0.724 | 3.81 |
| $C_6H_{14}O_2$ | 1,2-Diethoxyethane | 85 | 0.869 | 3.90 |
| $C_4H_{10}O$ | Diethyl ether | 35 | 0.714 | 4.27 |
| $C_7H_8O$ | Anisol | 154 | 0.994 | 4.30 |
| $C_5H_{12}O$ | *tert*-Butyl methyl ether | 55 | 0.741 | 4.5[a] |
| $C_7H_{14}O_2$ | Pentyl acetate | 149 | 0.876 | 4.79 |
| $CHCl_3$ | Chloroform | 61 | 1.483 | 4.81 |
| $C_5H_{10}O_2$ | Propyl acetate | 102 | 0.888 | 5.62 |
| $C_5H_{10}O$ | Tetrahydropyran | 88 | 0.881 | 5.66 |
| $C_6H_{12}O_2$ | *tert*-Butyl acetate | 95 | 0.867 | 5.67 |
| $C_6H_5Cl$ | Chlorobenzene | 132 | 1.106 | 5.69 |
| $C_5H_{10}O_2$ | Ethyl propanoate | 99 | 0.892 | 5.76 |
| $C_4H_8O_2$ | Ethyl acetate | 77 | 0.900 | 6.08 |
| $C_5H_{10}O_2$ | Butyl formate | 106 | 0.889 | 6.10 |
| $C_4H_8O_2$ | Methyl propanoate | 80 | 0.915 | 6.20 |
| $C_2H_4O_2$ | Acetic acid | 118 | 1.045 | 6.20 |
| $C_4H_8O_2$ | Propyl formate | 81 | 0.906 | 6.92 |
| $C_5H_{10}O$ | 2-Methyltetrahydrofuran | 78 | 0.855 | 6.97 |
| $C_3H_6O_2$ | Methyl acetate | 57 | 0.934 | 7.07 |
| $C_6H_{14}O_3$ | Diethylene glycol dimethyl ether | 162 | 0.943 | 7.23 |
| $C_2H_3Cl_3$ | 1,1,1-Trichloroethane | 74 | 1.339 | 7.24 |
| $C_4H_{10}O_2$ | Ethylene glycol dimethyl ether | 85 | 0.869 | 7.30 |
| $C_4H_8O$ | Tetrahydrofuran | 65 | 0.889 | 7.52 |
| $CH_2Br_2$ | Dibromomethane | 97 | 2.497 | 7.77 |
| $C_2H_2Cl_8$ | 1,1,2,2-Tetrachloroethane | 131 | 1.541 | 8.50 |
| $C_3H_6O_2$ | Ethyl formate | 54 | 0.917 | 8.57 |
| $CH_2Cl_2$ | Methylene chloride | 40 | 1.327 | 8.93 |
| $C_2H_4O_2$ | Methyl formate | 32 | 0.974 | 9.20 |
| $C_6H_4Cl_2$ | *o*-Dichlorobenzene | 180 | 1.306 | 10.12 |
| $C_8H_{18}O$ | 1-Octanol | 195 | 0.826 | 10.3 |
| $C_2H_4Cl_2$ | 1,2-Dichloroethane | 84 | 1.235 | 10.42 |
| $C_7H_{16}O$ | 1-Heptanol | 176 | 0.822 | 11.75 |
| $C_7H_8O$ | Benzyl alcohol | 205 | 1.042 | 11.92 |
| $C_4H_{10}O$ | Isobutanol | 108 | 0.802 | 12.47 |
| $C_6H_{14}O$ | 1-Hexanol | 158 | 0.814 | 13.03 |
| $C_5H_5N$ | Pyridine | 115 | 0.982 | 13.26 |
| $C_5H_{12}O$ | 3-Pentanol | 116 | 0.820 | 13.35 |
| $C_4H_{10}O_2$ | 2-Ethoxyethanol | 135 | 0.930 | 13.38 |
| $C_6H_4F_2$ | 1,2-Difluorobenzene | 94 | 1.160 | 13.38 |
| $C_5H_8O$ | Cyclopentanone | 131 | 0.949 | 13.58 |
| $C_5H_{12}O$ | 2-Pentanol | 119 | 0.809 | 13.71 |
| $C_4H_6O$ | Cyclobutanone | 99 | 0.955 | 14.27 |
| $C_5H_{12}O$ | 1-Pentanol | 138 | 0.814 | 15.13 |
| $C_5H_{10}O_3$ | Ethyl lactate | 155 | 1.033 | 15.4 |
| $C_5H_{10}O$ | 2-Pentanone | 102 | 0.809 | 15.45 |
| $C_5H_{10}O$ | 3-Pentanone | 102 | 0.810 | 17.0 |
| $C_3H_8O_2$ | Ethylene glycol monomethyl ether | 124 | 0.965 | 17.2 |
| $C_4H_{10}O$ | 2-Butanol | 100 | 0.806 | 17.26 |
| $C_4H_{10}O$ | 1-Butanol | 118 | 0.810 | 17.84 |
| $C_4H_{10}O$ | 2-Methyl-1-propanol | 108 | 0.802 | 17.93 |

**Table 2** (*Contd.*)

| Sum formula | Name | Bp/°C | Density $\delta$/g ml$^{-1}$ | Dielectric constant $\varepsilon$ |
|---|---|---|---|---|
| $C_5H_{10}O$ | Cyclopentanol | 140 | 0.949 | 18.5 |
| $C_4H_8O$ | 2-Butanone | 80 | 0.800 | 18.56 |
| $C_5H_9N$ | Pentanenitrile | 141 | 0.801 | 20.04 |
| $C_3H_8O$ | 2-Propanol | 82 | 0.781 | 20.18 |
| $C_3H_4O$ | Propargyl alcohol | 114 | 0.948 | 20.8 |
| $C_3H_8O$ | 1-Propanol | 97 | 0.780 | 20.8 |
| $C_3H_6O$ | Acetone | 56 | 0.785 | 21.01 |
| $C_4H_{10}O_2$ | 1,2-Butanediol | 191 | 1.002 | 22.4 |
| $C_6H_{14}O_4$ | Triethylene glycol | 285 | 1.127 | 23.69 |
| $C_3H_7NO_2$ | 1-Nitropropane | 131 | 0.996 | 24.7 |
| $C_4H_7N$ | 1-Butanenitrile | 118 | 0.794 | 24.83 |
| $C_2H_6O$ | Ethanol | 78 | 0.789 | 25.3 |
| $C_6H_{14}O_2$ | 2-Methyl-2,4-pentanediol | 197 | 0.923 | 25.86 |
| $C_7H_5N$ | Benzonitrile | 191 | 1.009 | 25.9 |
| $C_5H_{12}O_2$ | 1,5-Pentanediol | 239 | 0.991 | 26.2 |
| $C_3H_7NO_2$ | 2-Nitropropane | 120 | 0.982 | 26.74 |
| $C_3H_8O_2$ | 1,2-Propylene glycol | 188 | 1.036 | 27.5 |
| $C_2H_3F_3O$ | 2,2,2-Trifluoroethanol | 74 | 1.384 | 27.68 |
| $C_4H_{10}O_2$ | 1,3-Butanediol | 208 | 1.005 | 28.8 |
| $C_5H_{11}NO$ | *N*,*N*-Diethylformamide | 178 | 0.908 | 29.6 |
| $C_3H_5N$ | Propionitrile | 97 | 0.782 | 29.7 |
| $C_4H_{10}O_3$ | Diethylene glycol | 246 | 1.120 | 31.82 |
| $C_4H_{10}O_2$ | 1,4-Butanediol | 235 | 1.017 | 31.9 |
| $C_5H_9NO$ | *N*-Methylpyrrolidone | 202 | 1.023 | 32.6 |
| $CH_4O$ | Methanol | 65 | 0.791 | 33.0 |
| $C_3H_8O_2$ | 1,3-Propylene glycol | 214 | 1.054 | 35.1 |
| $C_6H_5NO_2$ | Nitrobenzene | 211 | 1.204 | 35.6 |
| $C_2H_3N$ | Acetonitrile | 82 | 0.786 | 36.64 |
| $CH_3NO_2$ | Nitromethane | 101 | 1.137 | 37.27 |
| $C_3H_7NO$ | *N*,*N*-Dimethylformamide | 153 | 0.944 | 38.25 |
| $C_4H_9NO$ | *N*,*N*-Dimethylacetamide | 165 | 0.937 | 38.85 |
| $C_2H_6O_2$ | Ethylene glycol | 197 | 1.114 | 41.4 |
| $C_3H_8O_3$ | Glycerol | 290 | 1.261 | 46.53 |
| $C_2H_6OS$ | Dimethyl sulfoxide | 189 | 1.101 | 47.24 |
| $H_2O$ | Water | 100 | 0.998 | 80.1 |
| $C_3H_7NO$ | *N*-Ethylformamide | 198 | 0.955 | 102.7 |
| $CH_3NO$ | Formamide | 220 | 1.133 | 111 |
| $C_2H_5NO$ | *N*-Methylformamide | 200 | 1.011 | 189 |

[a] There are isolated publications (*e.g.* M. Mirmehrabi and S. Rohani, *J. Pharm. Sci.*, 2005, **94**, 1560–1576) that report a dielectric constant of 2.60 for methyl *tert*-butyl ether. We took the value reported in the following two references: I. M. Smallwood, *Handbook of Organic Solvent Properties*, 1996; M. Winterberg, E. Schulte-Körne, U. Peters and F. Nierlich, *Ullmann's Encyclopedia of Industrial Chemistry*, 2010, Weinheim, Wiley-VCH. This value was confirmed by a recent remeasurement (Personal communication, Peter Nothhaft, Evonik, 2010). The newest version of the MTBE guide book (incl. appendices) does not list the value of dielectric constant (European Fuel Oxygenates Association, 2005).

have been observed, especially with alcohols. Nevertheless, the order in which the solvents are presented in Table 2 may help in both cases where a different solvent from either the same or a different class is wanted with a similar solubilising property.

## 2. Crystallisation by the layering technique

This technique normally requires more time than the vapour diffusion technique. Unlike an evaporation experiment, it is difficult to alter the course of a layering experiment, once it has been started. Well-suited containers for this type of experiment are NMR-tubes or other thin glass tubes. It is also possible to make such a device from a Pasteur pipette that has been sealed with a flame at its tip. All these tubes are inferior to flat bottom vials because an optical inspection of the crystals is very difficult due to the curved shape of the tube. If crystals sometimes form at the bottom of the tube, or fall down during attempts to retrieve them, inspection with the help of a microscope is almost impossible. Also, if small crystals have formed at the bottom of the tube, one may have to cut the tube in order to gain easy access to the crystals. Therefore, we use the least expensive NMR tubes available. The compound to be crystallised is dissolved at an almost saturated concentration in the denser solvent, which will form the lower layer. Especially, when compounds containing heavy elements are investigated, these compounds increase the density of the formed solution. In such cases, it is highly recommended that one carefully covers this solution with a middle layer consisting of the same solvent but without any added compound. This will create a buffer zone against the upper antisolvent. Finally, the antisolvent is added now (Fig. 1, right). All liquids should be slowly pipetted with extra-long Pasteur pipettes, which are commercially available or can be made with the help of a Bunsen burner. This will prevent any unwanted solvent mixing that immediately might lead to the formation of precipitates. In general, similar solvent pairs to those recommended for the vapour diffusion experiments are advisable. However, their

densities should be quite different and their boiling points should be rather high thereby preventing unwanted evaporation. As an example, we recommend di-*n*-butyl ether (bp 141 °C) as an ideal replacement for diethyl ether (bp 35 °C).

### 3. Crystallisation from gels

Crystallisation of small molecules has been described for aqueous[28] and even for non-aqueous gels made from polyethylene glycol.[29,30] This technique is extremely useful for the *in situ* synthesis and crystallisation of insoluble complexes.[28] However to the best of our knowledge, most of these approaches require 100 mg or more of the substance which additionally were never recovered after an unsuccessful attempt.[31] Otherwise, one could imagine utilizing gels for either the vapour or layering technique. Thereby, one would dissolve the analyte in the gel and continue with the experiments as usual. The advantage of using a gel might be a reduction of the number of nucleation events. Therefore this technique may be recommended when otherwise too many small crystals have formed and substantial amounts of material are available. As a side remark, proteins, which barely migrate out of a gel, have been successfully crystallised within gels.[32–35]

### 4. Two selected examples

First, we present an example, in which single crystals of excellent quality were grown by applying some of the initial solvent combinations mentioned in Table 1. Crystals of diethyl-4,4′-dipyridin-4-yl-2,2′-bipyrrole-3,3′-dicarboxylate (**1**)[36] suitable for single X-ray analysis were obtained by vapour diffusion from either THF against cyclohexane or methylene chloride against cyclopentane (Table S1†). In this chapter, the solvent is always mentioned first, then the anti-solvent. An ORTEP representation of **1** is shown in Fig. 2. The two central pyrrole rings are perfectly coplanar, between them there is an inversion centre. A hydrogen bridge is formed between N1 and the symmetry generated O13.

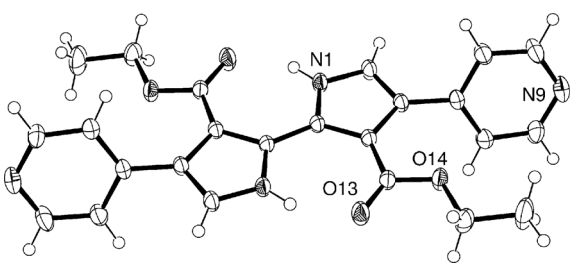


**Fig. 2** ORTEP representation of **1** at 50% probability.

In order to illustrate the optimisation of the single crystal growth by vapour diffusion, we will describe now our efforts to obtain single crystals of 3-carbethoxyquinoline (**2**). Compound **2** (ref. 37) was obtained as a minor side product of the homocoupling reaction of 3-carbethoxy-4-chloro-quinoline to yield diethyl-4,4′-biquinoline-3,3′-carboxylate.[38] Its appearance after recrystallisation from hexane has been described as needles.[39] Initial tests of vapour diffusion with THF against cyclohexane resulted in crystals that were too small for X-ray analysis (Fig. 3, upper left). The use of chloroform against cyclohexane gave better shaped crystals, but they were still too thin (Fig. 3, upper right) to be analyzed. Optimization of crystal growth by varying the chlorinated solvents (and adjusting the aliphatic antisolvent for an appropriate boiling point as discussed before) was eventually successful: vapour diffusion of trichloroethylene against heptane yielded plates (Fig. 3, lower left)

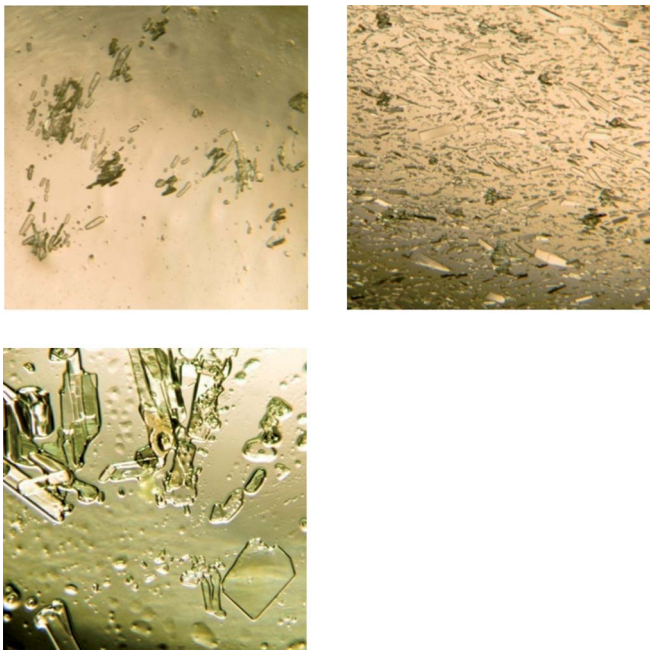

**Fig. 3** Crystals of **2** grown from tetrahydrofuran against cyclohexane (upper left), chloroform against cyclohexane (upper right), and trichloroethylene against heptane (lower left). The solvent is always mentioned first, the anti-solvent second. All images are on the same scale and the lengths of the image sections correspond to 1 mm each.

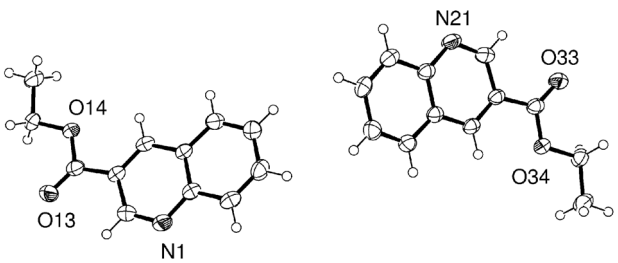


**Fig. 4** ORTEP representation of the asymmetric unit of **2** at 50% probability.

whose single crystal structure could be determined (Table S1†), despite the fact that we just used a relatively weak molybdenum sealed tube to generate the X-ray beam. 3-Carbethoxyquinoline (**2**) was crystallised in the triclinic space group $P\bar{1}$ with two molecules in the asymmetric unit (Fig. 4). As can be seen in Fig. 3a–c, crystallisation only took place upon complete evaporation of the solvents/antisolvents. Nevertheless, a strong dependence on the used solvents combination for the crystal formation exists. We were able to measure powder diffractograms of the latter two crystalline materials grown from either chloroform/cyclohexane or trichloroethylene/heptane. Visually, the diffractograms are very similar (Fig. S1†).

## Conclusions

This highlight is intended as an introduction for the beginner in the field of single crystal growth and as an inspiration for the more advanced researcher when their usual single crystal growing methods fail to work. We have described and critically compared the vapour diffusion, evaporation, layering, and gel crystallisation techniques. The first one is a flexible method which even allows the modification of an ongoing crystallisation experiment. We have listed a limited set of solvent/antisolvent combinations that we found useful for initial tests. If crystallisation experiments with a solvent/antisolvent pair will yield a crystalline material that is unsuitable for single crystal analysis, we recommend systematically exploring the most promising looking solvent/antisolvent class, including some of the less commonly used solvents. The provided table with 107 solvents and their most important properties shall help researchers to eventually grow single crystals.

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Electronic Supplementary Information (ESI) for:

# Some thoughts about the single crystal growth of small molecules

**Bernhard Spingler,* Stephan Schnidrig, Ferdinand Wild**

## Experimental methods

Most chemicals were purchased from Sigma-Aldrich. Tetrahydropyran and 1,2-difluorobenzene were from ABCR. Tablet tubes (40 mm x 12.75 mm) were from Müller-Krempel (Switzerland). Glass scintillation vials (20 ml, 61 mm x 28 mm) were from Wheaton.

Single crystal data were collected at 183(2) K on an Oxford Diffraction Xcalibur system with a Ruby detector using Mo $K_\alpha$ radiation ($\lambda$ = 0.7107 Å) that was graphite-monochromated. A suitable crystal was covered with oil (Infineum V8512, formerly known as Paratone N), mounted on top of a glass fibre and immediately transferred to the diffractometer. The program suite CrysAlis$^{Pro}$ was used for data collection, multi-scan absorption correction and data reduction.[1] The structures were solved with direct methods using SIR97[2] and refined by full-matrix least-squares methods on $F^2$ with SHELXL-97.[3] The final structures were checked for higher symmetry with help of the program Platon.[4] CCDC 849680 and 800843 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Powder X-ray diffraction (XRD) was performed at room temperature on a STOE STADI P diffractometer in transmission mode (flat sample holders, Ge monochromator and Cu $K_{\alpha1}$ radiation).

## Table S1: Crystallographic data for 1 and 2.

| Compound | **1** | **2** |
|---|---|---|
| Empirical formula | $C_{24}H_{22}N_4O_4$ | $C_{12}H_{11}NO_2$ |
| Space group | $P2_1/c$ | P-1 |
| a [Å] | 9.3329(2) | 7.5975(5) |
| b [Å] | 12.7049(3) | 12.2026(7) |
| c [Å] | 9.1195(2) | 12.8137(8) |
| $\alpha$ [°] | 90 | 61.607(6) |
| $\beta$ [°] | 98.085(2) | 77.247(5) |
| $\gamma$ [°] | 90 | 78.353(5) |
| Volume [$Å^3$] | 1070.59(4) | 1012.74(11) |
| Z | 2 | 4 |
| Crystal size [$mm^3$] | 0.50 x 0.27 x 0.11 | 0.21 x 0.13 x 0.08 |
| Independent reflections | 3660 [R(int) = 0.0209] | 5457 [R(int) = 0.0402] |
| Reflections observed (>2sigma(I)) | 3116 | 2329 |
| Completeness to theta | 99.9 % to 30.44° | 99.9 % to 29.13° |
| Max. and min. transmission | 0.9898 and 0.7360 | 0.9928 and 0.8558 |
| Data / restraints / parameters | 3660 / 0 / 146 | 5457 / 0 / 273 |
| Goodness-of-fit on $F^2$ | 1.032 | 0.810 |
| Final R indices (I>2sigma(I)) | R1 = 0.0464, wR2 = 0.1216 | R1 = 0.0479, wR2 = 0.0782 |
| Largest diff. peak and hole [$e.Å^{-3}$] | 0.405 and -0.223 | 0.163 and -0.268 |

Figure S1:

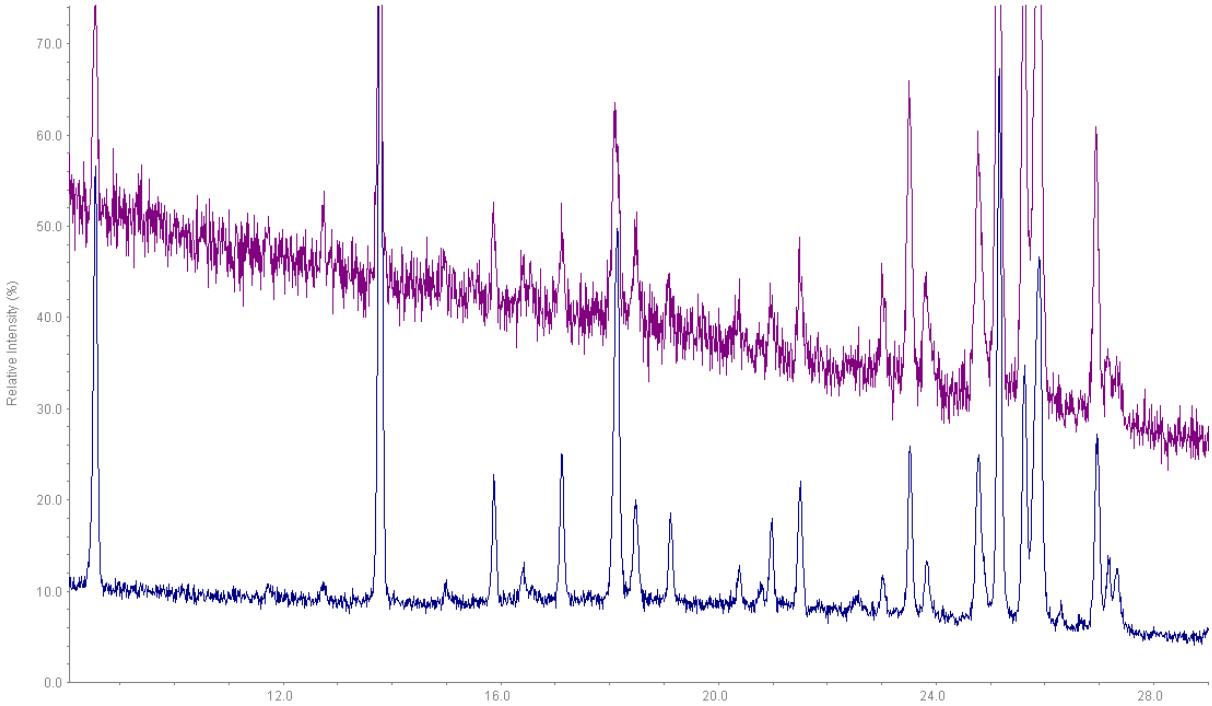


Fig. S1: Powder diffractograms of **2** grown from either chloroform against cyclohexane (top) or trichloroethylene against heptane (bottom).

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