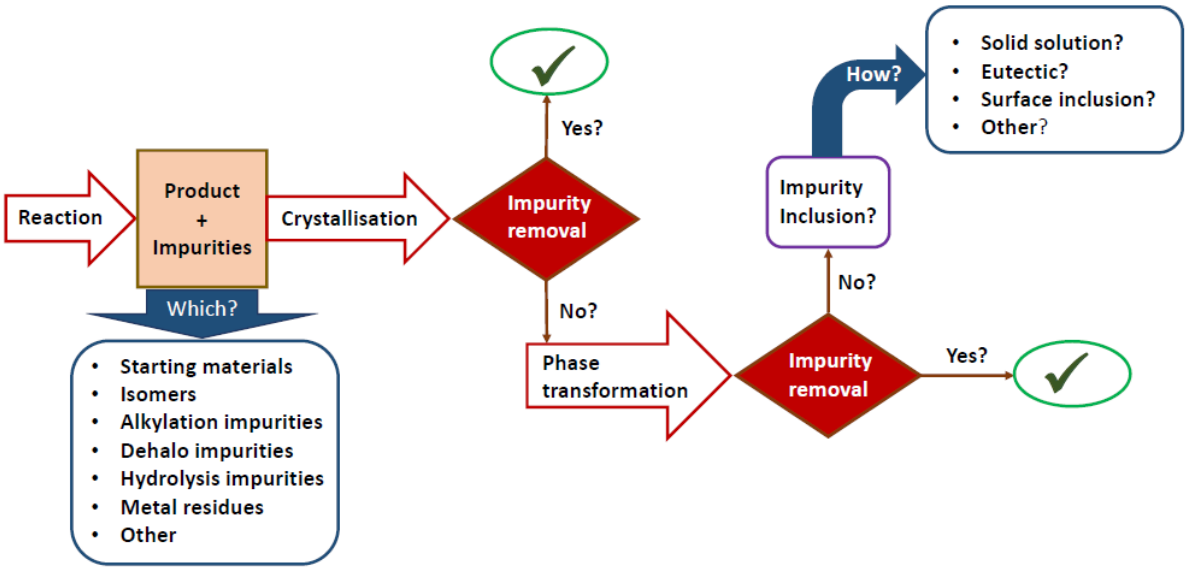
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Reaction
Product
+
Impurities
Crystallisation
Impurity
removal
Yes?
No?
Which?
Starting materials
Isomers
Alkylation impurities
Dehalo impurities
Hydrolysis impurities
Metal residues
Other
Phase
transformation
Impurity
removal
Yes?
No?
Impurity
Inclusion?
How?
Solid solution?
Eutectic?
Surface inclusion?
Other?

Impurity Occurrence and Removal in Crystalline Products from Process Reactions

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Department of Chemistry / Analytical and Biological Chemistry Research Facility / Synthesis and Solid-state Pharmaceutical Centre, University College Cork, Cork, Ireland.

ABSTRACT The behaviour of impurities when subjected to crystallisations, and related processes such as recrystallisation and reslurrying, has been reviewed with a particular focus on the years 2000 to 2015, but also including significant cases from outside that period. Small molecule pharmaceuticals and similar small organic molecules are included but not biomolecules, inorganics or minerals. Phase impurities are only covered when a phase transformation is involved with the management of an impurity. Introductory examples illustrating some general features of crystallisation as a method of purification are presented, as well as approaches to quantifying the effectiveness of purification. The review classifies cases based on the behaviour of the specific impurities covered. The classes of behaviour observed are removal by washing, recrystallisation or reslurrying (Class 1), impurities not being removed by these operations (Class 2), and impurities which are removed in conjunction with a phase transformation (Class 3). Examples of each of these types of behaviour are presented, with many processes producing impurities which fall into more than one of these classes. Studies on the inclusion of extraneous molecules into crystalline materials are also covered. These particularly include incorporation of compounds as solid solutions, but also

eutectic formation and inclusion at surfaces during crystal growth. The relationship between types of impurities and behaviour during processing is also examined.

KEYWORDS Pharmaceuticals, impurities, crystallisation, solid solutions

1. Introduction

Generation of impurities during process chemistry, and the retention or otherwise of these during crystallisation and subsequent processing, is part of the reality of manufacturing pharmaceuticals and similar fine chemicals.[1,2] FDA guidelines[3-6] designate organics, inorganics and residual solvents as likelys impurities, and notes that organic impurities can arise during manufacturing or storage from starting materials, by-products, intermediates, degradation products, reagents, solvents, ligands, catalysts, stereoisomers and filter aids. Actual or potential impurities can arise during synthesis, purification and storage, and these must be noted and listed in the specification for any new drug substance. Of particular concern are possible genotoxic impurities such as alkyl halides or sulfonates.[7,8] Organic impurities with a close structural similarity to the pharmaceutical entity are referred to as related substances.[9]

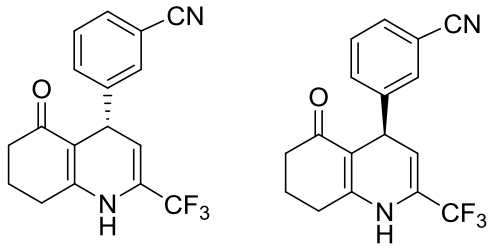
Crystallisation is probably the most important method of product isolation and purification in pharmaceutical manufacturing. Hence, the presence of impurities in the crystallisation medium or in the crystal product is clearly a significant issue which may affect the specification compliance of the batch. Any impurities present must be known, quantified and shown to be below specified limits. It is widely appreciated that impurities in the crystallisation medium can affect crystal nucleation and growth rates, crystal phase occurrence including polymorphism or solvate formation, and crystal morphology including habit and crystal size distribution.[10-16] The behaviour of impurities when subjected to crystallisations has been less explicitly studied, despite the critical role crystallisations play in

impurity management. This review examines literature reports on the impact of crystallisations, and related processes such as recrystallisation and reslurrying, on the levels of specific impurities. The review covers the years 2000 to 2015 inclusive plus some significant earlier or more recent cases. The focus is on 'small molecule' pharmaceuticals and other drug-like small molecules, especially with regard to cases in which impurities arise from specific process chemistry. The scope of the review excludes proteins and other biomolecules, as well as minerals and bulk inorganics. Also excluded are phase impurities such as crystal polymorphs, solvates or hydrates, except in cases where phase transformations have been associated with control of specific molecular impurities. An overview of the behaviour of impurities when subjected to solid state processes such as recrystallisation or reslurrying should highlight patterns which can assist in designing a general scheme for troubleshooting impurities in solids and supporting rational process design.

1.1 Crystallisation as a method of purification

The success of crystallisation from solution as a method of purification lies in the selectivity of the processes of crystal nucleation and growth for the components of the crystal lattice, while other components present in the system, i.e. impurities, selectively remain in the mother liquor. This contrasts with, for example, spray drying, in which the solvent is fully removed to leave the solutes in particle form with no selection between differing components. The relative quantities of crystallising compound and impurities present in solution and the yield of the crystallisation are key factors in achieving successful purification. For example, in the crystallisation of ZD0947 **1**, the enantiomer **2** was the significant impurity (Figure 1). Crystallisations of compound **1** from acetonitrile were found to correspond closely to ideal behaviour characterised by the van't Hoff equation, giving a 90% yield upon cooling from 77

to 5 °C. The input material needed to be of sufficient enantiopurity such that conditions for the crystallisation of racemic crystals did not occur. That required consideration of the yield of crystallised compound and the composition of the remaining solutes, such that the latter remained below the solution eutectic composition for formation of the crystalline racemic compound.[17]

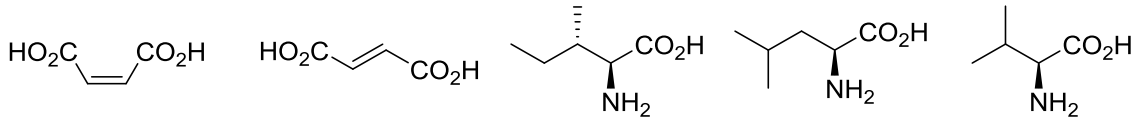


1 **2**

Figure 1. ZD0947 and Enantiomer

A similar point is illustrated by the example of maleic acid **3** (Figure 2), in which fumaric acid **4** can occur as an impurity (Figure 2). The water solubility of fumaric acid is considerably lower than that of maleic acid (0.8 g/100 g and 91.2 g/100 g, respectively, at 30 °C), such that the presence of undissolved fumaric acid impurity can lead to significant underestimation of the solubility of maleic acid.[18] The eutectic for this system at 30 °C is at ca. 99.2% maleic acid : 0.8% fumaric acid, and the impurity, fumaric acid, is the less soluble compound. Hence, formation of solid impurity can occur if the impurity concentration of the input material is greater than the eutectic composition. Similarly, in the crystallisation of L-isoleucine **5** (Figure 2), separate crystals of the impurities L-leucine **6** and L-valine **7** (Figure 2) could form as a physical mixture with L-isoleucine crystals, if the impurity level was sufficiently high.[19] For example, solutions containing between 5 to 10 mol L-Leu **6** per 100 mol L-isoleucine **5** gave crystals with a mixture of needle-like and hexagonal habits, with a greater proportion of the crystals of hexagonal habit being observed with greater quantities of

L-leucine impurity. High quantities of L-valine impurity gave samples containing agglomerates that consisted of well formed needles of L-isoleucine around which smaller crystals were present, believed to be of L-valine.

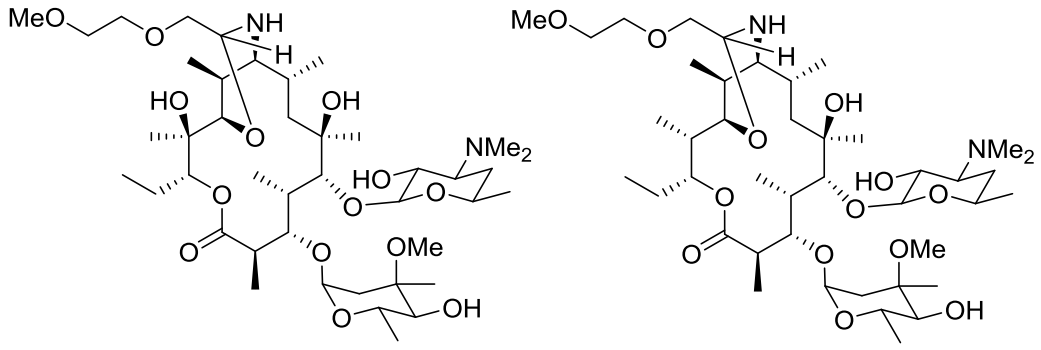


3 **4** **5** **6** **7**

Figure 2. Structures of Maleic Acid, Fumaric Acid, L-Isoleucine, L-Leucine and L-Valine

1.2 Quantification of purification

Workers have sought to quantify the level of impurity reduction in crystallisations. For example, impurity levels of dirithromycin **8** (Figure 3) following recrystallisation from acetone/water was plotted as a function of impurities in the technical material, showing a reasonable correlation for both overall impurities and dirithromycin B impurity **9** specifically.[20]

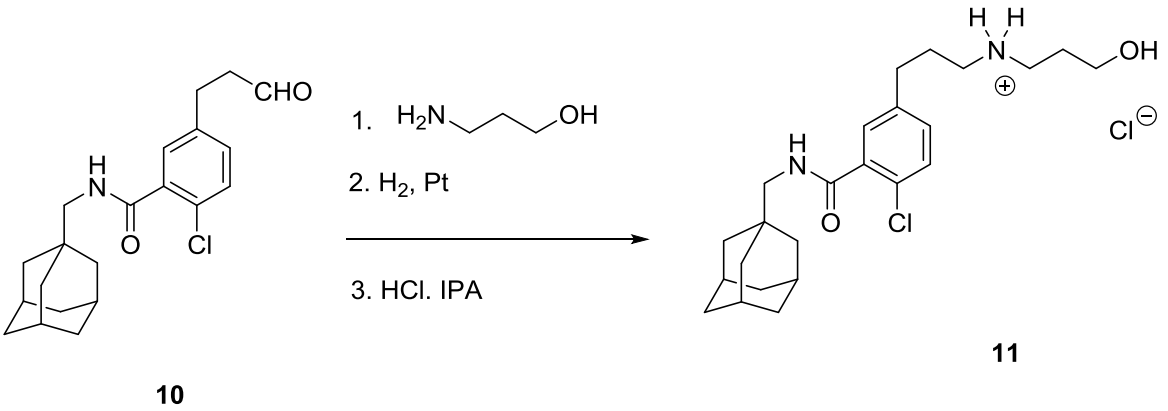


8 **9**

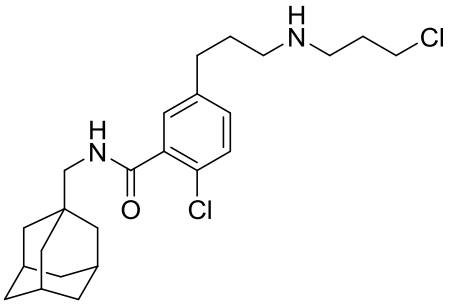
Figure 3. Dirithromycin and Dirithromycin B

Purge factors for impurities have also been developed. These attempt to quantify the ease of removal of impurities during process development in general, rather than specifically by crystallisation.[21, 22] In the purge factor approach, assessment of potential carry-over of impurities, especially genotoxic impurities, is based on factors such as reactivity, solubility, volatility, ionisability, and additional physical processes which may be involved, such as chromatography. Solubility relates to solubility in the solvent system used during isolation, in general by crystallisation. A score is assigned based on the physicochemical properties of the impurity relative to the process conditions. These are multiplied together to give the purge factor. For solubility, which is the most important of these factors relating to carry-over of impurities in crystallisation, an impurity which is freely soluble in the solvent system is assigned a value of 10, a moderately soluble impurity is assigned 3, a sparingly soluble impurity assigned 1. If the impurity in question is highly soluble it will remain within the mother liquors and be purged from the crystallized product, and hence have a high purge factor. For example, in the formation of AZD9056 **11** by reductive amination followed by isolation by hydrochloride salt formation, a chloride by-product **12** occurs as an impurity (Scheme 1). The calculated purge factor for this impurity based on the aforementioned principles was 3, indicating that the process had limited capacity to remove the impurity and that the extent of its formation needed to be controlled. In this case, the experimental purge factor was found to be more favourable. In the same process, isopropyl chloride was also formed by degradation of the solvent, however, the calculated purge factor for this was 10, based on high solubility and volatility, predicting that isopropyl chloride should be effectively removed.[21, 22]

Scheme 1. Final Steps in a Route to AZD9056



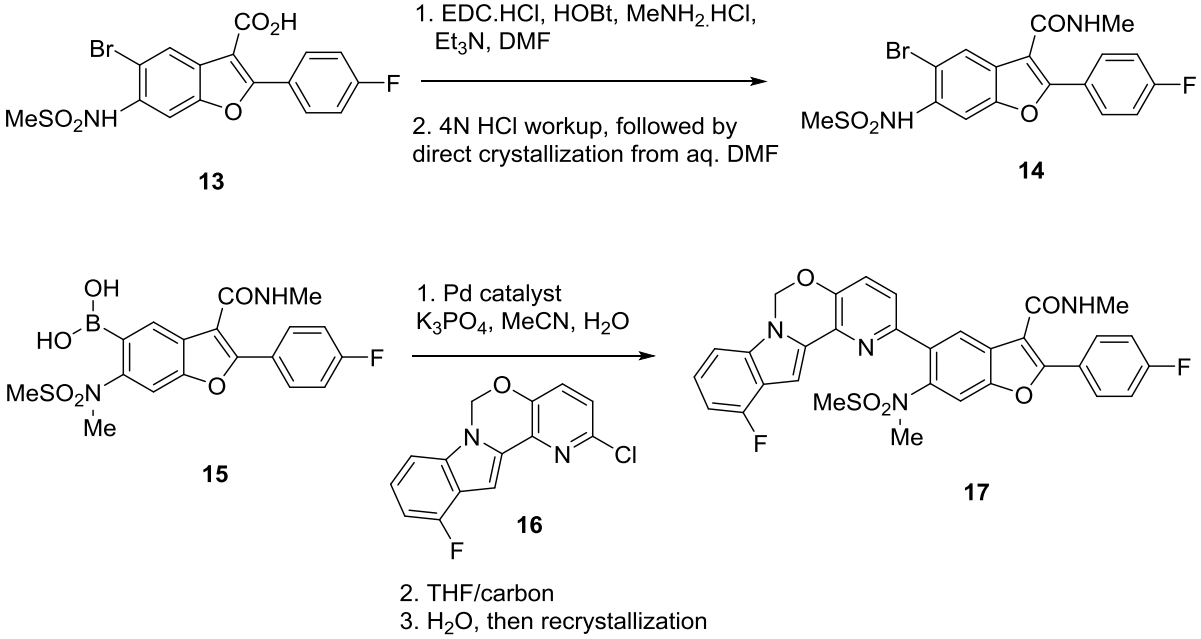
10 **11**



12

A further example of the use of purge factors concerned the synthesis of an *N*-methylamide **14** as an intermediate in a route to hepatitis C virus treatment MK-8876 **17** (Scheme 2). Compound **14** was obtained by coupling using EDC with direct crystallisation of the product. The purge factor for EDC was 10. During a subsequent coupling step to give **17**, the purge factor for the starting arylboronic acid **15** was found to be 10 for crystallisation.[23]

Scheme 2. Route to MK-8876



13 14 15 16 17

1.3 Ordering of the review material

Published work on impurities in pharmaceutical or related solids broadly cover two approaches both of which are represented in this review. The first are cases of occurrence and/or removal of impurities in crystallisations and related processes drawn from reports of process chemistry and process development. The second are studies on the inclusion of impurities in solids, especially in crystal lattices. Many of the latter involve solid solutions. These two approaches rarely overlap, hence understanding of how impurities form part of the composition of solids obtained by process crystallisations is limited.

Scheme 3 summarises the common relationships between processes such as reactions and crystallisations, the generation of products and impurities, and the removal of the latter. The scheme is based on generalisations from the reported literature on impurities in pharmaceutical and fine chemical manufacturing over the review period. A number of reports of impurity removal in conjunction with a phase transformations were noted. This approach is particularly appropriate if the crystal lattice of the initial crystal form is insufficiently

discriminating in the rejection of impurities. In such cases, a different crystal lattice may be more discriminating.

In line with the trends observed in Scheme 3, the review will cover cases which fall under the following general classes.

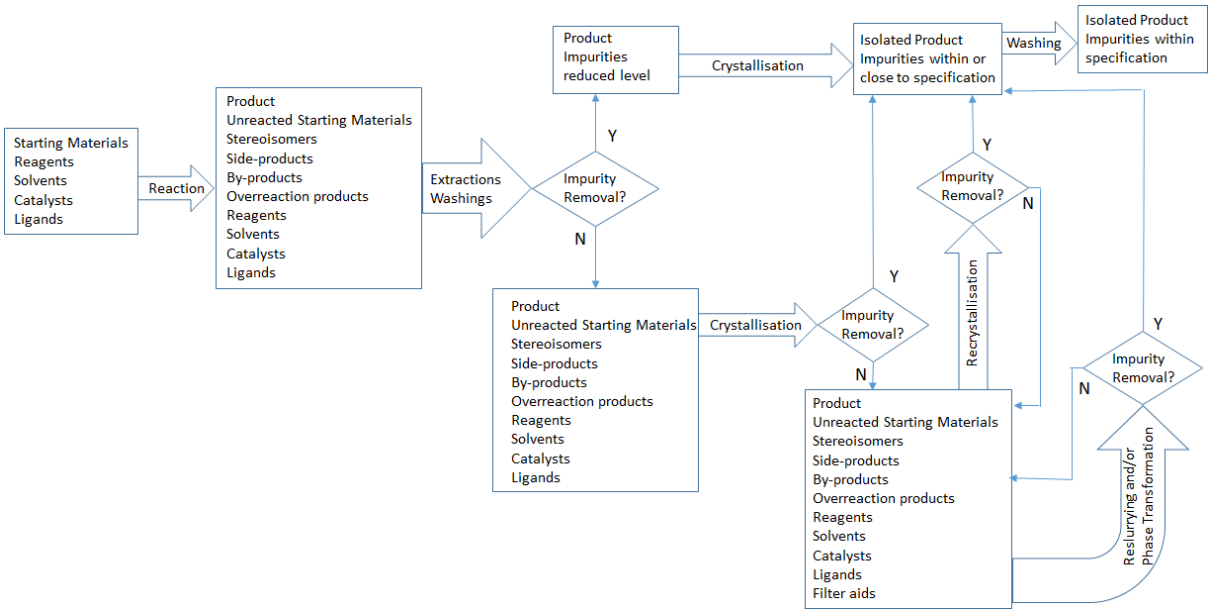
- Class I: Impurities which are satisfactorily removed by recrystallisation.
- Class II: Impurities which are not satisfactorily removed by recrystallisation.
- Class III: Impurities which are removable in conjunction with a phase transformation.

In the next section, cases from the literature will be described, sub-divided according to how they fall into the above classes. Note that any individual process may display more than one class of behaviour, i.e. may have impurities which were acceptably removed by crystallisation, (Class I) or were not so removed (Class II), or required a phase transformation (Class III). The literature cases studies are therefore presented in the following order: Class I behaviour only, Class II behaviour only, Class I and II behaviour, Class III behaviour only, Class I and III behaviour, Class II and III behaviour, and Class I, II and III behaviours. Information on the cases reviewed is taken as reported and were not evaluated for how extensive the process development was for any specific case.

The review will examine incorporation of impurities within crystal lattices and at crystal growth steps. Formation of solid solutions is clearly an important possibility in cases in which impurities are not removed by crystallisation, but other modes of inclusion or absorption need also be considered, examining factors such as variation on crystal growth and morphology, surface adherence and washing efficiency. An overview of the material also suggested that particular process types were particular prone to generate of impurities, for

example alkylation reactions. We, therefore, also examined the correlation between the processes for removing impurities and the reactions which generate them.

Scheme 3 Schematic of general relationships between reaction process, products and impurities.



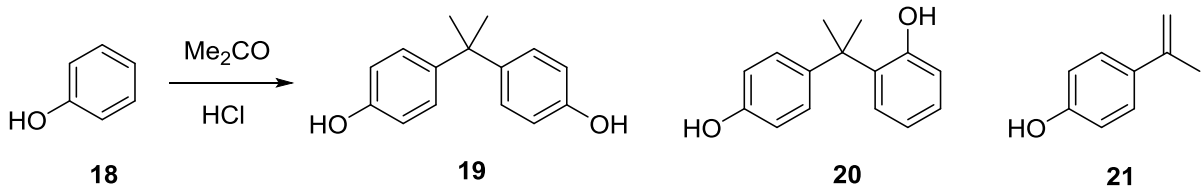
2. Impurity occurrence in crystallisations in process chemistry

2.1 Class I only

In manufacturing scale crystallisations, impurities are often adsorbed on crystal surfaces and can be removed by efficient washing. An example is provided by the manufacture of bisphenol A **19** by condensation of phenol **18** and acetone,[24,25] giving rise to a wide variety of impurities, including unreacted phenol **18**, 2',4''-dihydroxyl-2,2-diphenylpropane **20**, isopropenylphenol **21** and many others (Scheme 4).[26] One process for the crystallisation of bisphenol A gave product which was found to be 99.5% pure, with the main source of

impurities being mother liquor adhering on crystal surfaces. The temperature stages of the crystallisation protocol were altered to reduce the amount of fine crystals and increase the average crystal size, allowing for improved separation of crystal product from the mother liquor, giving material of 99.8% purity. Further recrystallisation was found to improve the quality of the bisphenol A product up to 99.99%.[27]

Scheme 4. Production of Bisphenol A

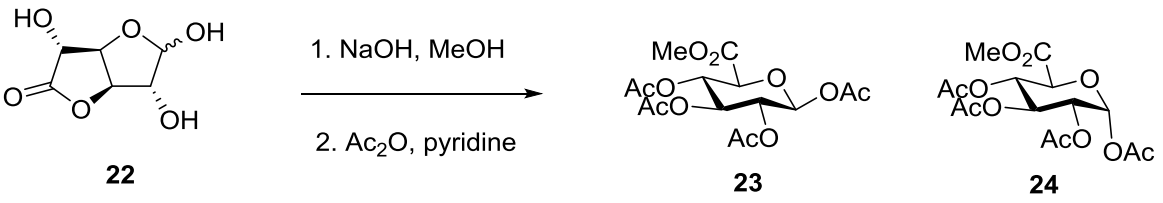


18 **19** **20** **21**

Methyl tetra-*O*-acetyl-β-D-glucopyranuronate **23** has been very widely used as a protected intermediate in the synthesis of glucuronides.[28-30] Compound **23** is prepared from D-glucurono-6,3-lactone **22** by reaction with methanol and sodium hydroxide followed by either acetic anhydride in pyridine or acetic anhydride with perchloric acid (Scheme 5).[31] The product is isolated by cooling of the final reaction mixture from which the product crystallises. Processing of the mother liquors has provided the α-anomer **24**, i.e. both the α and β anomers are formed.[32] For example, in one operation of the process, the required β-anomer **23** was obtained as a crystalline solid in 39% yield, while the mother liquor was subjected to silica gel chromatography to provide the α-anomer **24** as a crystalline solid in 37% yield. HPLC analysis of batches of the β anomer **23** showed 2.5 to 3.0% of the crystallised material to be the α anomer **24**. No α anomer **24** was detected in any of the recrystallised batches of β anomer **23**. Thorough washing of the directly obtained β anomer

23 with methanol was also found to remove completely the α anomer **24**, i.e., impurity **24** can be removed by efficient washing.[33]

Scheme 5. Preparation of Methyl Tetra-*O*-acetyl-D-glucopyranuronate

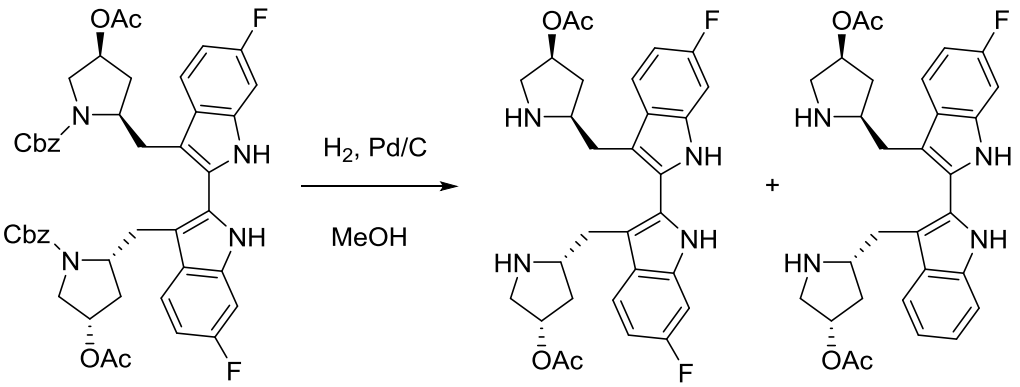


22 **23** **24**

2.2 Class II only

In the synthesis of birinapant to support clinical trials as a treatment of cancer and HBV, removal of carbobenzyloxy groups by hydrogenation was required in the synthesis of intermediate **26** (Scheme 6). In addition to the required product **26**, the des-fluoro product **27** was also obtained in 1.0 to 1.5 % quantities. Compound **27** could not be removed by recrystallization and was carried though to subsequent steps.[34] Use of HBr/HOAc as alternative to hydrogenation for removal of the carbobenzyloxy groups avoided the problem.[34,35]

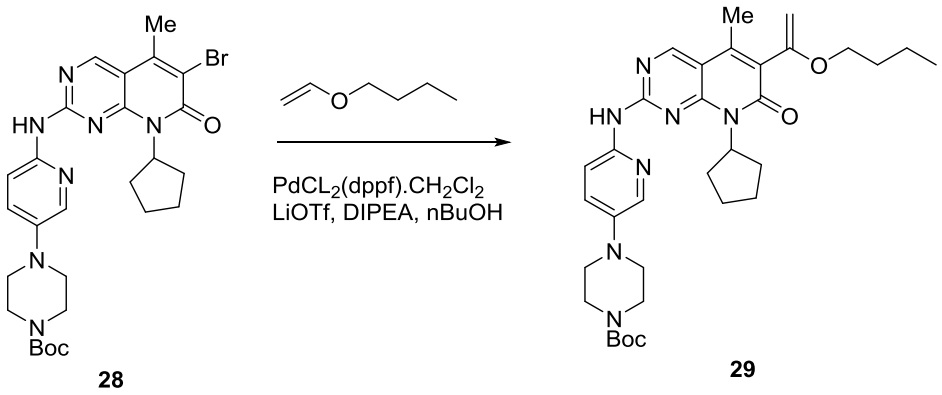
Scheme 6. Deprotection by Hydrogenation Step in a Route to Birinapant



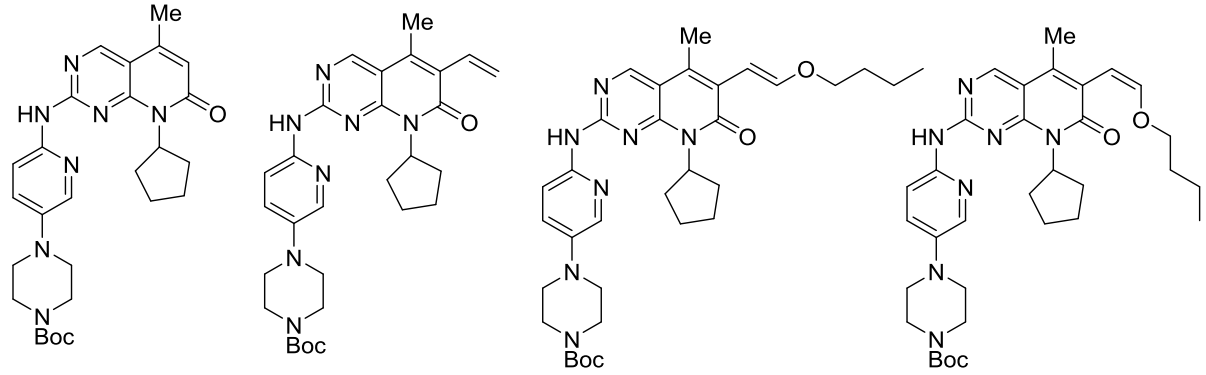
25 **26** **27**

During process development for the manufacture of palbociclip, a Heck coupling step to obtain the intermediate **29** gave rise to impurities **30**, **31**, **32** and **33** (Scheme 7). Isolation of the required product **29** by addition of water/*n*BuOH, filtration, addition of water/diaminopropane (to scavenge palladium residues), separation of the organic layer, seeding, cooling from 60 to 20 °C, and washing of the isolated solid with *n*BuOH and MTBE gave compound **29** with the impurities **30**, **31**, **32** and **33** present in quantities from 0.1 to 1.0%.[36, 37] Replacement of catalyst and ligand with $Pd(OAc)_2$ and DPEPhos reduced levels of all impurities to below 0.05%, except for the des-bromo impurity **30**, which was still over 0.5%. Optimising reaction temperature, catalyst loading, catalyst to ligand ratio and controlling water content also minimised impurities. Filtration and washing with aqueous 1,2-diaminopropane were effective in reducing palladium residues to below 200 ppm.[36]

Scheme 7. Coupling Step in a Route to Palbociclip



28 29

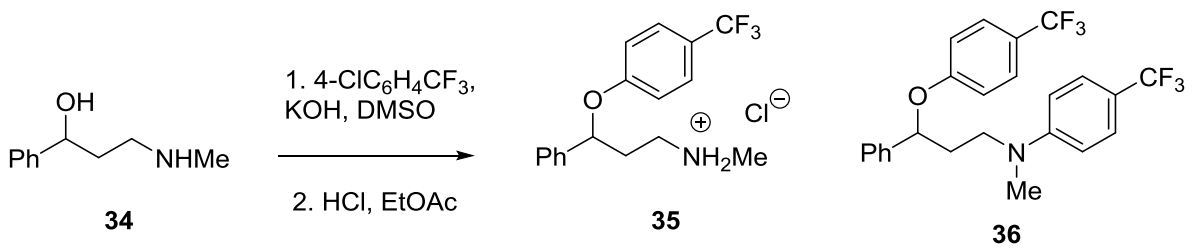


30 31 32 33

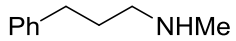
2.3 Class I and Class II

Several routes to the antidepressant fluoxetine hydrochloride **35** are known, many of which involve 3-methylamino-1-phenylpropanol **34** as an intermediate product (Scheme 8).[38,39] This can be converted into fluoxetine hydrochloride by reaction with 4-chloro(trifluoromethyl)benzene. The hydrochloride can be crystallised by addition of anhydrous HCl to a solution of the product free base in ethyl acetate, with recrystallisation from, for example, acetone, giving typically 80% yield of **35** by cooling crystallisation. Several impurities were observed, including unreacted 3-methylamino-1-phenylpropanol **34** and the *N,O*-diarylated derivative **36**, however, both are reduced to less than 0.1% by the recrystallisation step.[40]

Scheme 8. 4-Chloro(trifluoromethyl)benzene Route to Fluoxetine.HCl



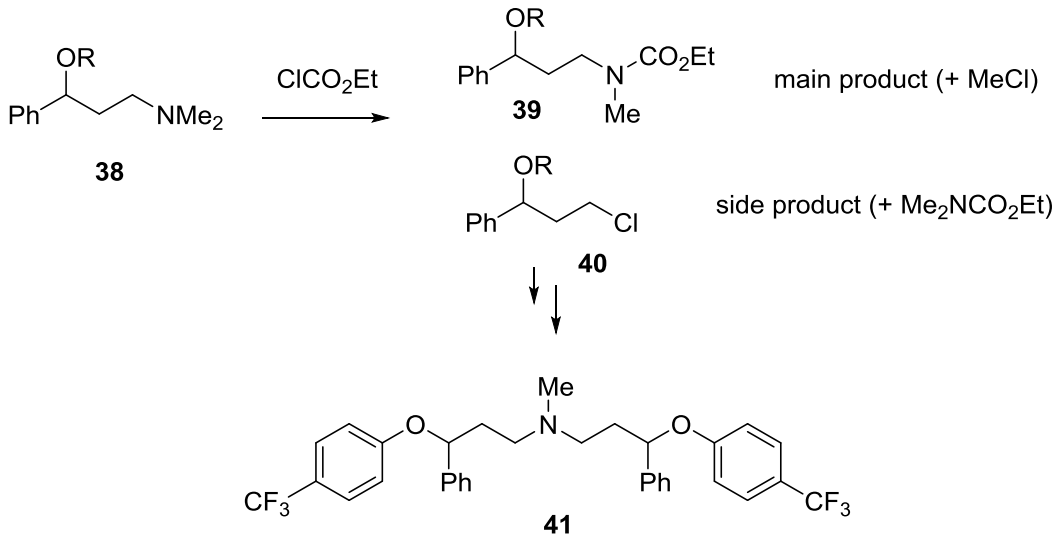
Other impurities were found in routes to fluoxetine hydrochloride **35** which displayed Class II behaviour. For example, many routes to **35** involve a reductive step, using reagents such as B_2H_6, $NaBH_4$, H_2/Pd-C, $LiAlH_4$ or Zn/AcOH, giving compound **37** (Figure 4) as an impurity formed by removal of the benzylic hydroxyl group.[39] Many routes also involve a late stage von Braun demethylation using ethyl chloroformate (Scheme 9).[41] Under these conditions, a chloride side-product **40** can also form, along with *N*,*N*-dimethylethylcarbamate, which can be subsequently alkylated to give the persistent impurity **41** in up to 3% depending on the precise route. For both the de-hydroxylated impurity **37** and impurity **41**, recrystallization of fluoxetine hydrochloride **35** from acetone can fail to remove up to half of the impurity.[40]



37

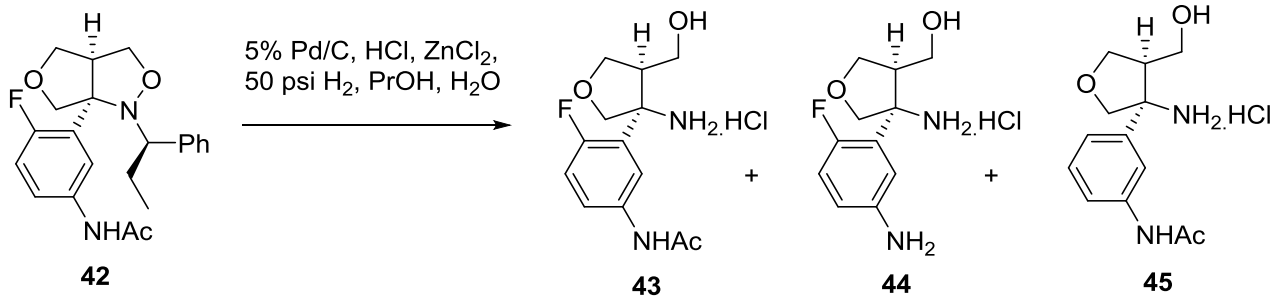
Figure 4. Reduction Step Impurity from Fluoxetine.HCl Syntheses

Scheme 9. Von Braun Demethylation in Fluoxetine.HCl Synthesis



A hydrogenation step leading to an intermediate **43** in the synthesis of beta-amyloid cleaving enzyme inhibitor LY2886721 gave two impurities including a deacetylated derivative **44** which was adequately rejected by crystallisation from water and 1-propanol (Scheme 10).[42] By contrast, a des-fluoro impurity **45** was less well rejected. It was necessary to supress formation of impurity **45** to <0.1% by addition of acid to the process, at the cost of increased but manageable quantities of the deacetylated impurity **44**.[42]

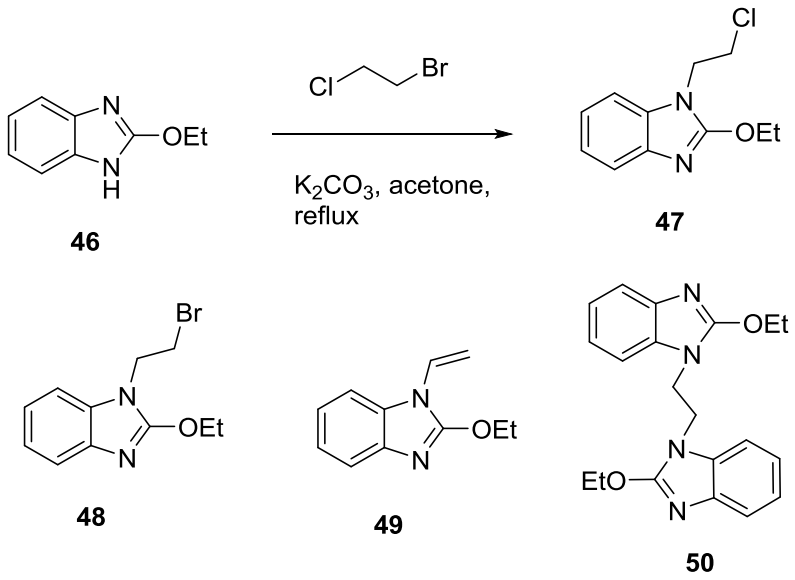
Scheme 10. Synthesis of an Intermediate in a Route to LY2886721



During development of a route to the serotonin receptor antagonist flibanserin, the intermediate **47** and impurities **48**, **49** and **50** were obtained from an alkylation process (Scheme 11). The vinyl impurity **49** and the dimeric impurity **50** were completely removed

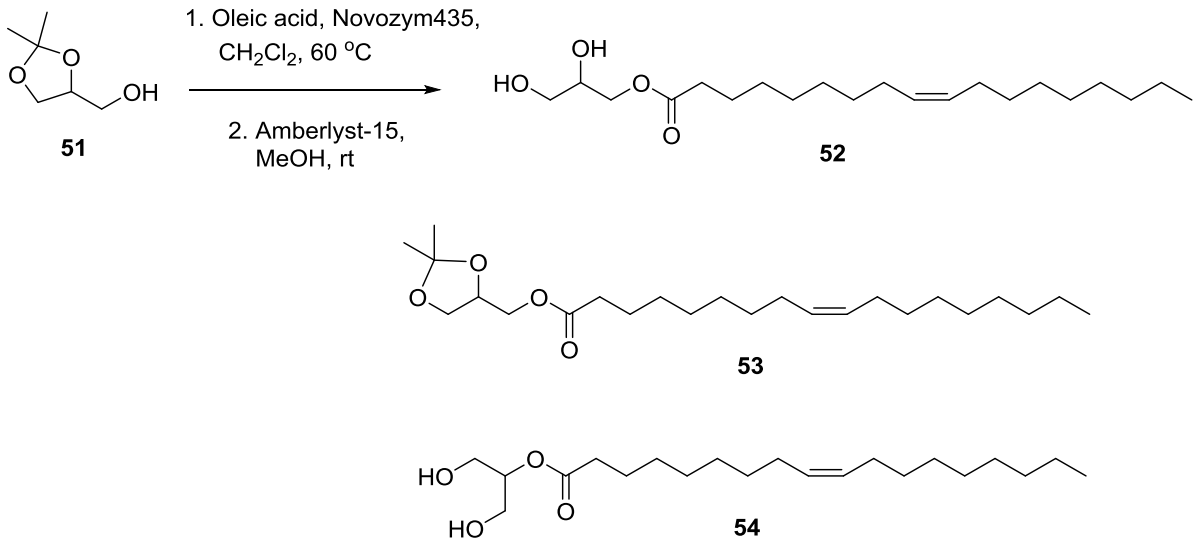
by recrystallisation from heptane. The bromo impurity **48** was not completely removed but was consumed in the next step of the process.[43]

Scheme 11. Alkylation Step in a Route to Flibanserin



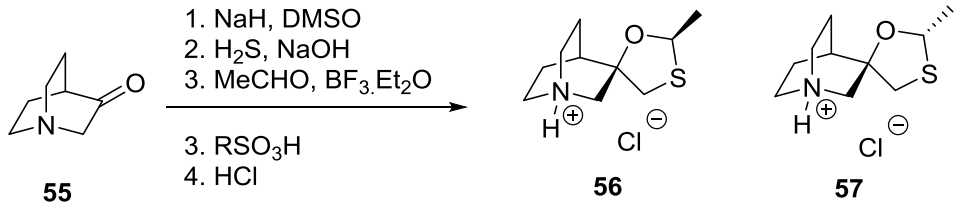
Manufacturing of 1-monoolein **52** by enzymatic acylation of glycerol-1,2-acetonide **51**, followed by hydrolysis using Amberlyst resin in methanol, was found to give a number of impurities. Recrystallisation from hexane at −30 °C over 5 hours was sufficient to remove methyl oleate, oleic acid and 1-monooleoyl-2,3-acetonide **53** impurities (Scheme 12). However, multiple recrystallizations were required to completely remove the 2-monooleate **54** and glycerol impurities.[44]

Scheme 12. Route to 1-Monoolein



Cevimeline **56**, a muscarinic receptor antagonist, is obtained by a route with gave a mixture of *cis* and *trans* isomers, in which the *cis* isomer **56** is the required API and the *trans* isomer **57** constitutes an impurity (Scheme 13).[45] Provided the *cis* isomer **56** is in excess, formation of a sulfonate salt gives a solid enriched in the *cis* isomer **56**, from which further formation and crystallisation of the hydrochloride salt gives the API in >99.5% purity.[46]

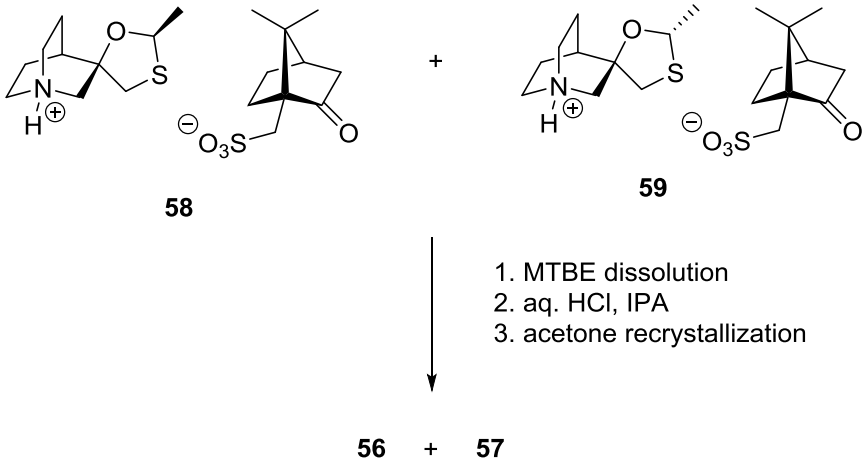
Scheme 13. Route to Cevimeline



Trans isomer **57** can be obtained as a reference standard, using the filtrate following crystallisation of the *cis* isomer **56**, as the camphorsulfonic acid (CSA) salt **58** (Scheme 14). Evaporation to dryness gives a mixture of the isomers as CSA salts, enriched in the *trans* isomer **59**. Re-dissolution in MTBE and crystallization of the HCl salt by addition of aq. HCl and IPA, followed by recrystallization from acetone, gave the hydrochloride salt **57** predominantly as the *trans* isomer (Scheme 14). This sequence reduced the cevimeline **56**

content from ca. 33% to ca. 4%, however preparative HPLC was necessary to obtained fully pure trans isomer **57**.[47]

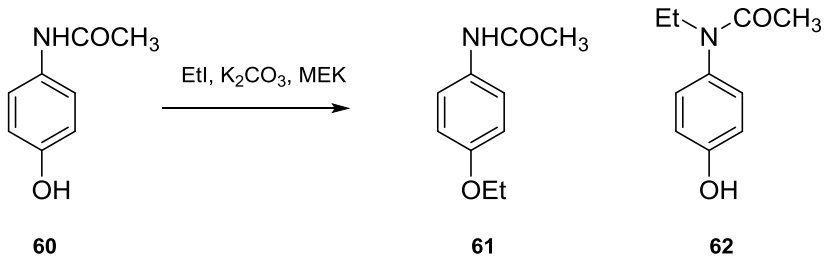
Scheme 14. Isolation of the *Trans* Isomer of Cevimeline



Phenacetin **61** was marketed as an analgesic and anti-pyretic drug before being withdrawn due to nephrotoxicity.[48] One preparation of phenacetin involves ethylation of 4-hydroxyacetamide **60** (i.e. paracetamol or acetaminophen) (Scheme 15).[49,50] This process gives unreacted **60** and the *N*-ethylated derivative **62** as impurities in quantities of up to 1%, with **62** as the major impurity. Impurity **62** is completed removed by recrystallisation, despite being the more abundant impurity, while starting compound **60** may not be completely removed after multiple recrystallizations.[51] Related substances such as 4-ethyloxyaniline (4-phenetidine) **63** (Scheme 16), a degradation product of phenacetin, was also found to be effectively excluded from phenacetin crystals (Supplementary Information). An amide C4 hydrogen bonded chain[52] is a key feature of the crystal structure of phenacetin.[53] As a tertiary amide, compound **62** cannot serve as a component of an amide C4 chain, which provides an attractive rationale for its poor inclusion into the phenacetin crystals (Scheme 16). As an aniline, 4-ethyloxyaniline **63** possess N-H bonds with can donate hydrogen bonds as part of a

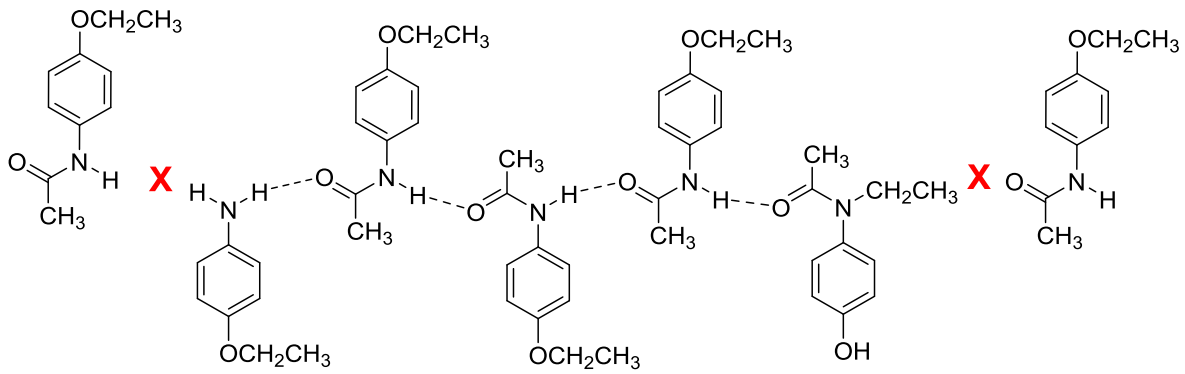
C4 chain, but lacks the carbonyl C=O bonds necessary to accept hydrogen bonds as part of such as chain (Scheme 16).

Scheme 15. Preparation of Phenacetin



60 **61** **62**

Scheme 16. Likely Discontinuity of the Phenacetin Amide C4 Chain by 4-Hydroxy-N-ethylacetanilide **62** and 4-Ethoxyaniline **63**

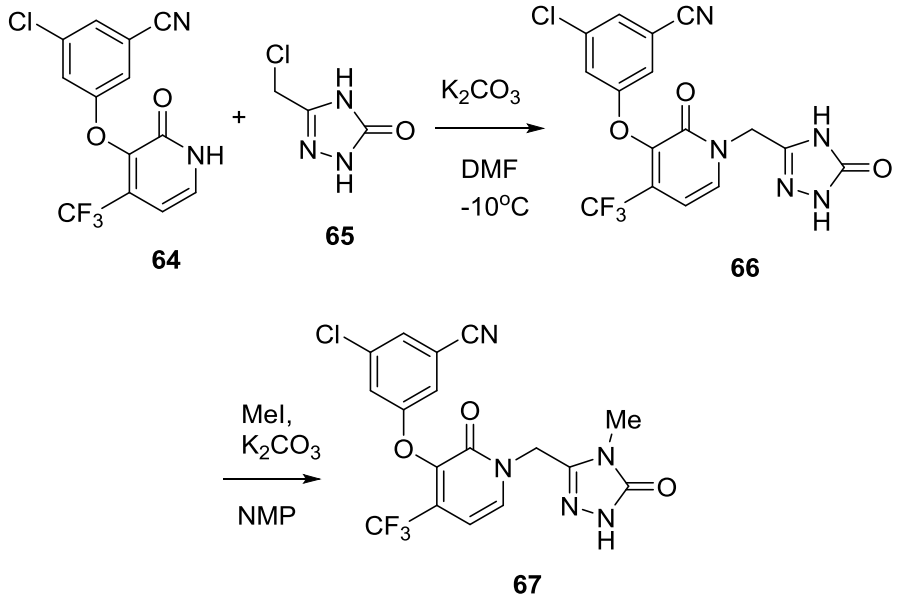


63 **62**

During development of a kilo-scale synthesis of doravirine **67**, des-methyl doravirine **66** could be obtained by reaction of the pyridone **64** and triazolinone chloride **65** (Scheme 17).[54] Recrystallization from acetonitrile/water give complete rejection of remaining triazolinone chloride **65**.[55] Subsequently methylation of des-methyl doravirine **66** gave doravirine **67**. After removal of the base by filtration, addition of water gave crude doravirine **67** including

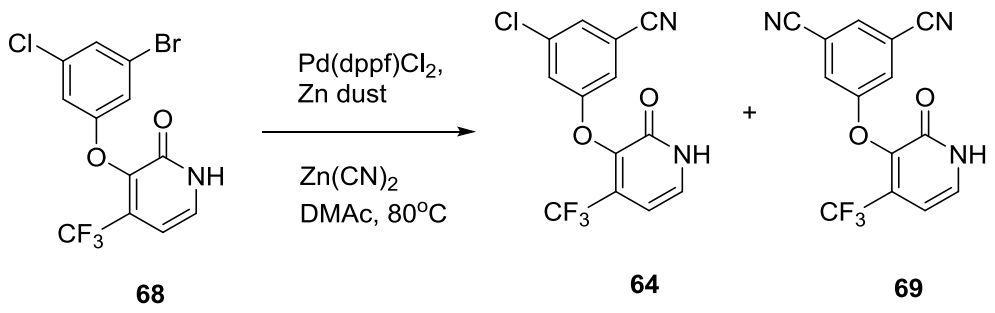
ca. 5% bis-methyled impurity and impurities methylated at alternative positions. Recrystallisation from NMP-ethanol sufficiently removed these impurities.

Scheme 17. Steps in a Synthesis of Doravirine



Synthesis of the cyano intermediate **64** also produced the bis(cyano) impurity **69**, which could not be adequately removed (Scheme 18). Investigation of THF, acetone, toluene, IPA, DMF/H_2O, acetonitrile, dioxane, 2-butanone and methanol as solvents for the crystallization of intermediate **64** gave 2.0 to 3.6% of impurity **69**.[54] This was avoided by altering the reaction conditions to CuCN in NMP, and use of the iodo analogue of the bromide **68**.[54,56]

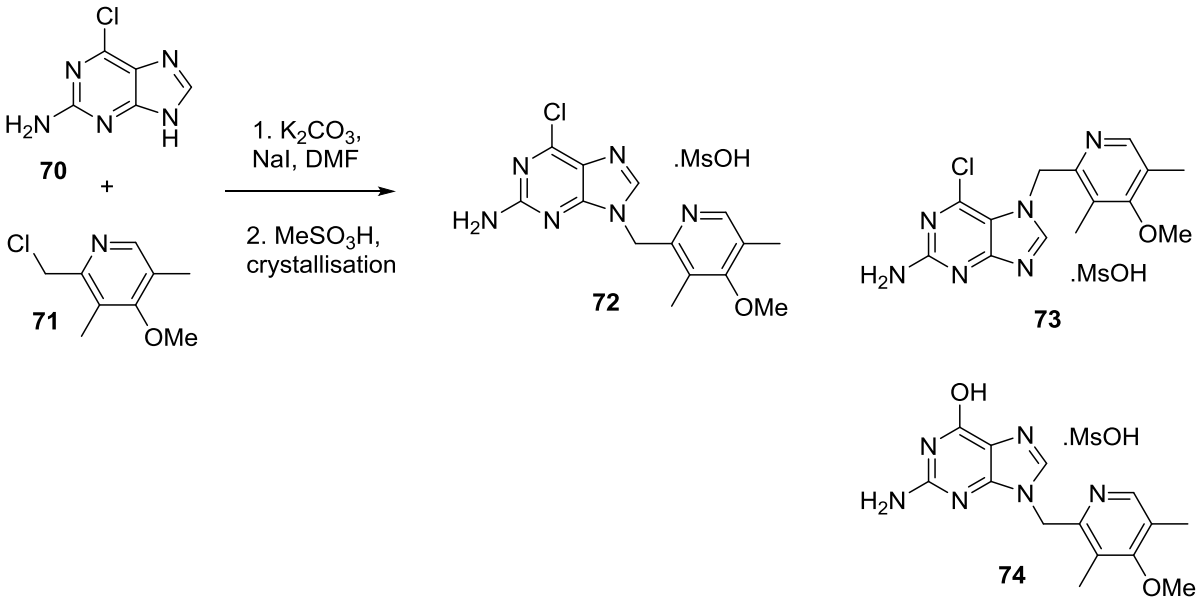
Scheme 18. Synthesis of an Intermediate in a Route to Doravirine



2.4 Class III only

The HSP90 inhibitor **72** (Scheme 19) below was obtained by alkylation at the N^9 position of chloropurine **70** followed by isolation of the mesylate salt. The main impurity observed was the N^7 alkylated isomer **73** which was found as a 1.4% impurity in the crude material. Crystallisation of the mesylate salt of **72** from DMSO by antisolvent addition also resulted in some formation of a hydrolysis product **74** in quantities of up to 0.4%. Use of methyl acetate as the anti-solvent gave material containing 0.45% of the N^7 alkylated impurity **73**, while use of ethyl acetate as antisolvent gave 0.28% of the same impurity. In the final developed process, the methylate salt **72** was dissolved in DMSO at 22 °C, ethyl acetate antisolvent was added with seeding and cooling to 0 °C to give the required product with both impurities under 0.06%.[57]

Scheme 19. Route to HSP90 Inhibitor



Three polymorphs of an unidentified ‘API X’ **75** (Figure 5) were known, designated forms A, B and C, of which the crystal structures and supramolecular packing motifs of these forms were fully characterised. A dimer **76** involving H···F hydrogen bonds was one of the motifs found in forms A and B. Compounds **77**, **78**, **79** and **80** had been found as process impurities and the impact of these on crystallisation of **75** was investigated. For example, it was found that impurities **77** and **78** inhibited the transformation of form A to form B at 30 ºC in IPA. Under these conditions, form B is the thermodynamically preferred form and batches of pure form A, or form A in the presence of impurities **79** or **80**, transform to form B. It was noted that impurities **77** and **78** possess the molecular groups necessary for formation of a dimer motif similar to **76**, whereas impurities **79** and **80** lack such groups. Impurities **79** or **80** can therefore mimic dimers **76** and affect the polymorphic transformation.[13]

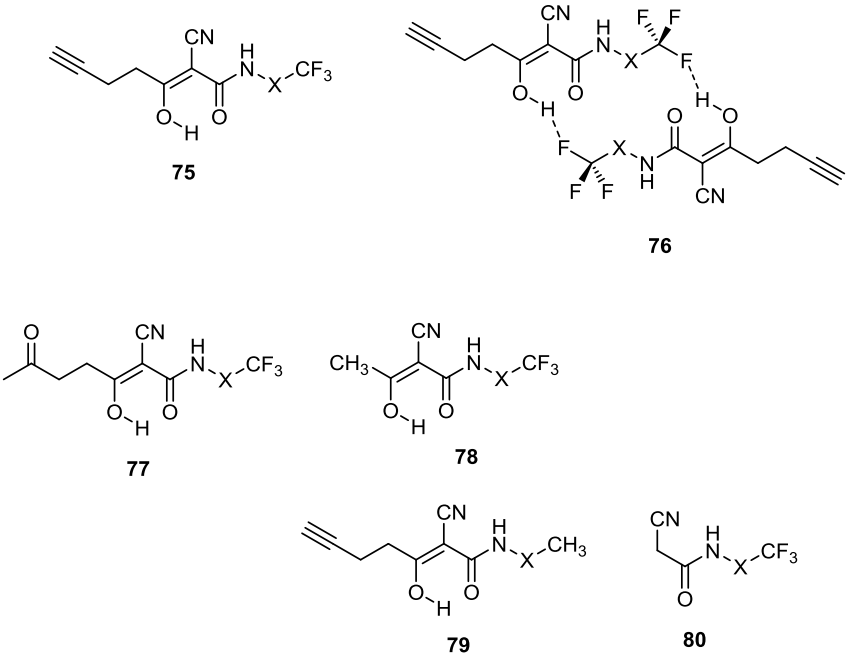
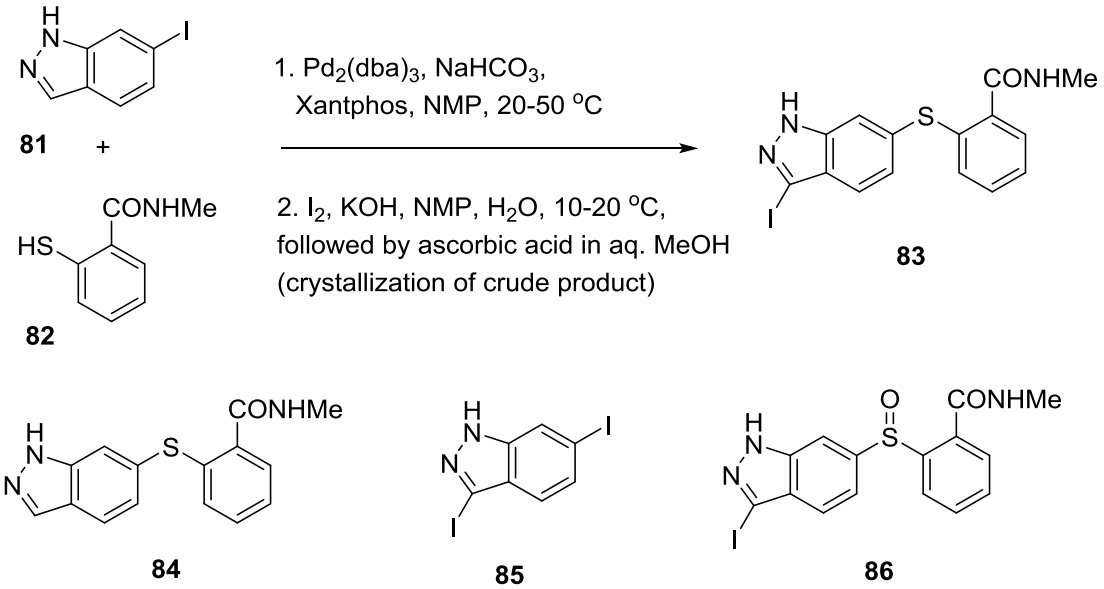


Figure 5. API 'X' and Impurities

2.5 Class I and Class III

In development of a manufacturing route to axinitib, a telescoped process was used to obtain the intermediate **83** (Scheme 20). Recrystallisation was necessary to control impurities **84**, **85** and **86**, present in quantities of 0.1% to > 2.0% in the crude material. Dissolution in NMP, with 1,2-diaminopropane to solubilize palladium residues, followed by addition of methanol, followed by water antisolvent, give the following acceptable levels of impurities **84**, **85** and **86** in quantities of 0.05% to 0.80%.[58]

Scheme 20. Synthesis of an Intermediate in a Route to Axinitib



Axinitib **87** itself (Figure 6) was found to be highly polymorphic with at least five anhydrous forms and many solvates. Compound **87** was obtained from a Heck coupling in NMP, after which the reaction mixture was diluted with THF containing 1,2-diaminopropane to solubilize palladium residues. The crude product was isolated as a THF solvate by cooling and water antisolvent addition. Recrystallization from NMP/THF with ethanol antisolvent addition, seeding and slow cooling gave axitinib **87** as the preferred anhydrous polymorph. The impurities found were compound **84** (0.21 – 0.30% in crude material), **88** (0.13 – 0.17%

in crude) and **89** (0.11 – 0.13% in crude). After crystallisation as described, these were all reduced to ≤0.05% and palladium levels below 7 ppm.[58]

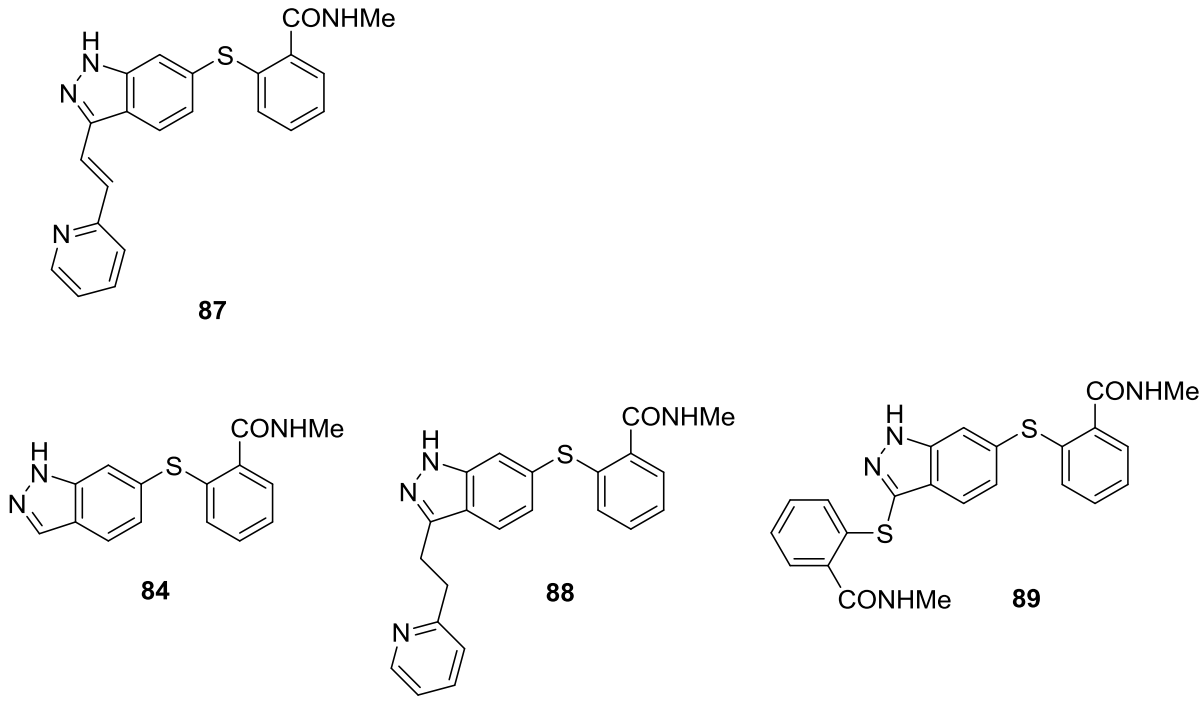
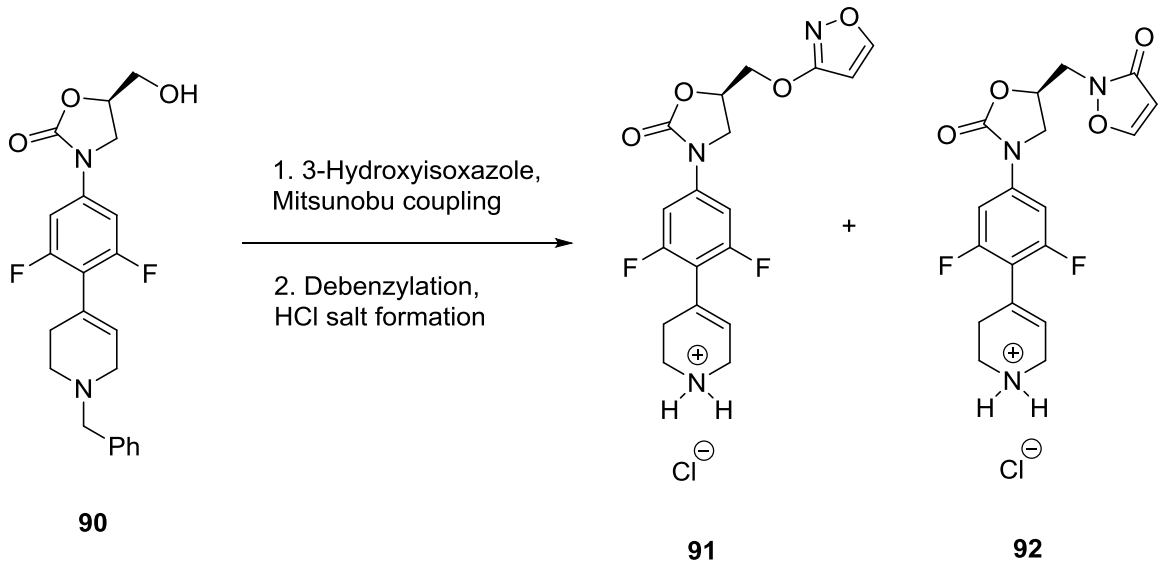


Figure 6. Axitinib and Impurities

In the development of a route to an oxazolidinone intermediate, a Mitsunobu coupling step was used to form both isoxazole and oxazolidone products, which, following debenzylation and hydrochloride formation gave **91** and **92** respectively, the latter as an impurity in up to 4.5% quantities (Scheme 21). Two crystal forms of oxazolidinone **91** were observed, a hydrate which occurred as needles and an anhydrous form which occurred in a morphology described as "lumps". It was found that the hydrate form displayed very good selectivity for rejection of impurity **92**, whereas the anhydrous form showed poor rejection, accommodating up to 4% of the impurity. Determination of the crystal structures of both forms showed that a key motif in the structure of the anhydrous form was a π–π interaction involving the central difluoroaryl and oxazolidinone rings of **91**, but not the terminal isoxazole and piperidine rings, which was consequently not discriminatory towards the isoxazolidinone ring of **92**. By

contrast, the structure of the hydrate contained a hydrogen bond between the water molecule and the ring oxygen of the isoxazole, which cannot be formed by impurity **92**. Hence, ensuring that **91** crystallised as the hydrate form gave material with quantities of impurity **92** in acceptable levels of ≤0.3%.[59]

Scheme 21. Synthesis of an Oxazolidinone Intermediate



2.6 Class II and Class III

During the process development for the manufacturing of S-2367 **93**, a neuropeptide Y receptor antagonist, an acid impurity **94** was found to be above specification (> 0.06%) in a pilot plant process (Figure 7). Studies on the recrystallisation of S-2367 **93** from aqueous acetone indicated that the acid **94** was forming as a hydrolysis degradation product during recrystallisation. Residual HCl in the batches for recrystallisation, arising from use of thionyl chloride in a previous step, promoted the hydrolysis of the amide group in **93**. Use of dry acetone gave poor throughput of material. Washing was found to be ineffective at removing the residual **94**, hence it was concluded that compound **94** was enclosed in the crystals of product **93**, possibly by the formation of an intermolecular interaction between the acidic

impurity **94** and the basic pyridine ring of S-2367 **93**, i.e. structure **95** in Figure 7. Repeated recrystallization was found to give further hydrolysis of **93**. It was noted that crude **93** was obtained as a metastable polymorph and that this could be converted to the stable polymorph by slurrying in aqueous acetone at 55 °C. This was also found to be a mild and effective way to remove impurity **94**. For this removal to be successful, it was necessary to maintain the crude product as the metastable form before slurrying, which was achieved by washing with water. However, it was also desirable to eliminate the need for the polymorphic transformation, hence it was found that **93** could be obtained as a stable polymorph within the specified purity directly by crystallisation provided the acid **94** was converted into its sodium salt.[60]

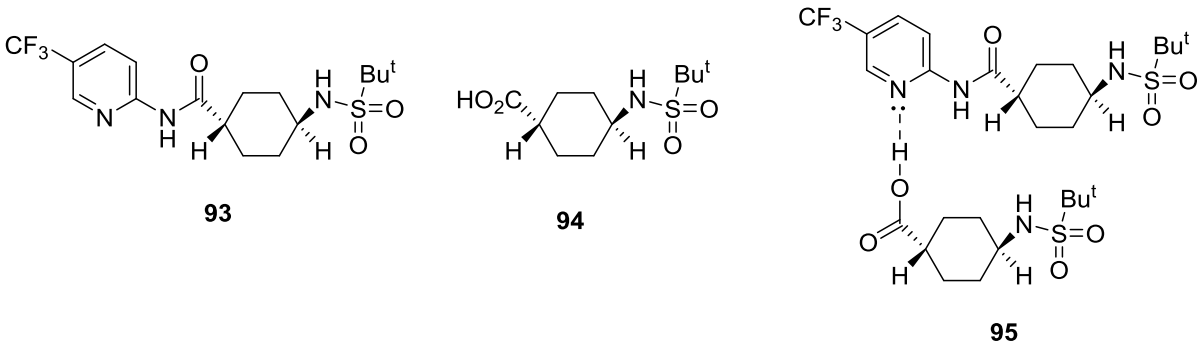
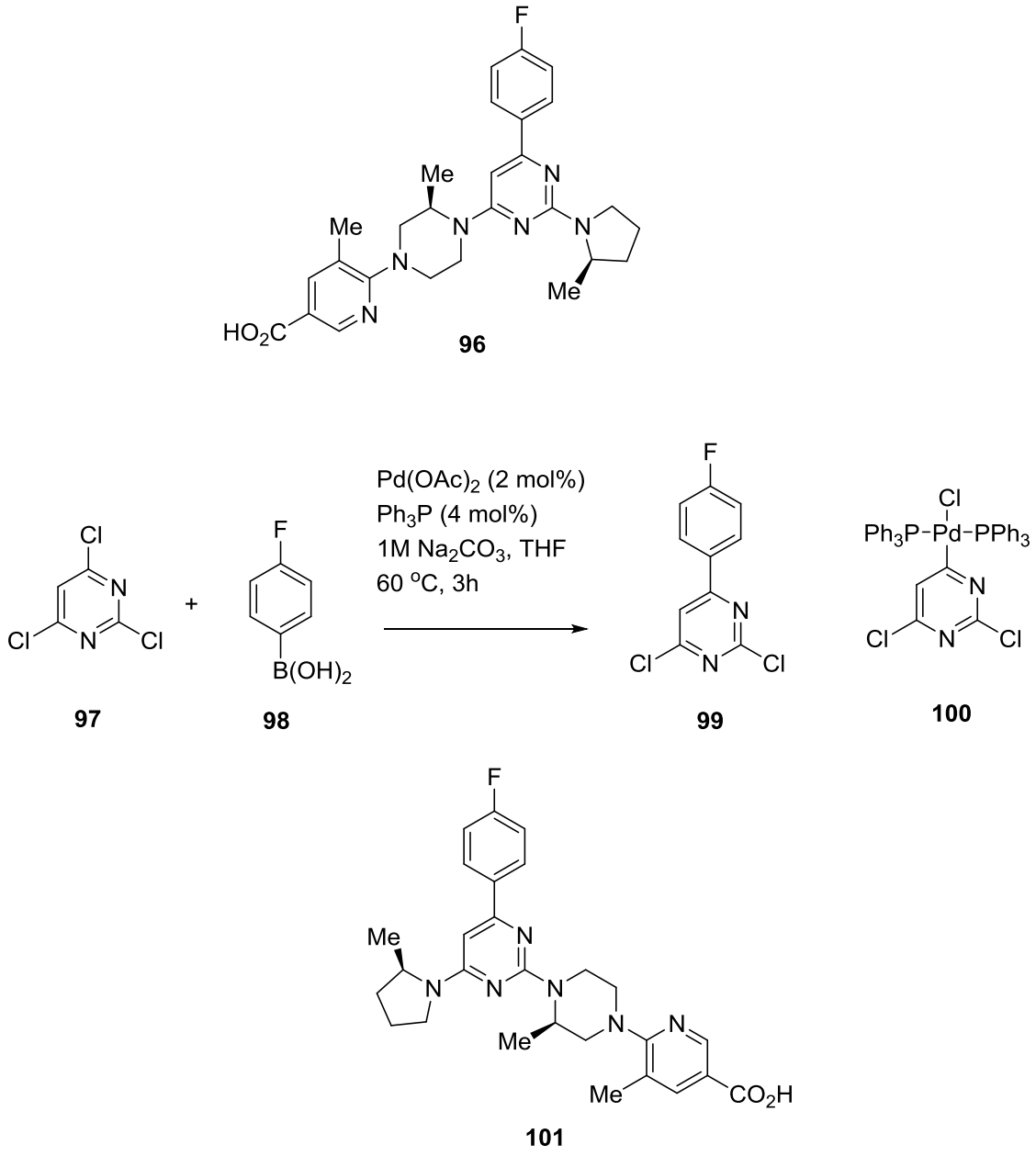


Figure 7. S-2367 and Degradation Impurity

During the process development of TRVP1 Antagonist **96**, the intermediate step shown involved crystallization of the product **99** from MTBE/heptane, from which the product was found to contain ca. 1.5 mol% of the palladium complex **100**. However, the presence of complex **100** proved beneficial in a subsequent step. The final product was found to contain the impurity **101** in 3% quantity carried through from previous steps in which it had not been rejected by crystallization by salt formation (Scheme 22).[61] However, target compound **96** was also found to form a crystalline DMSO solvate which rejected impurity **101**. Hence, the

aqueous reaction extract (after washing to remove other impurities) was extracted into MTBE and the solvent switched to DMSO, which resulted in a slurry of the DMSO solvate with stirring at room temperature. The solvate was isolated, dissolved in DMAc/water, aq. NaOH added and warmed to 45 °C to achieve dissolution, MsOH was added and cooled to crystallize the mesylate salt of **96** without impurity **101**.[61]

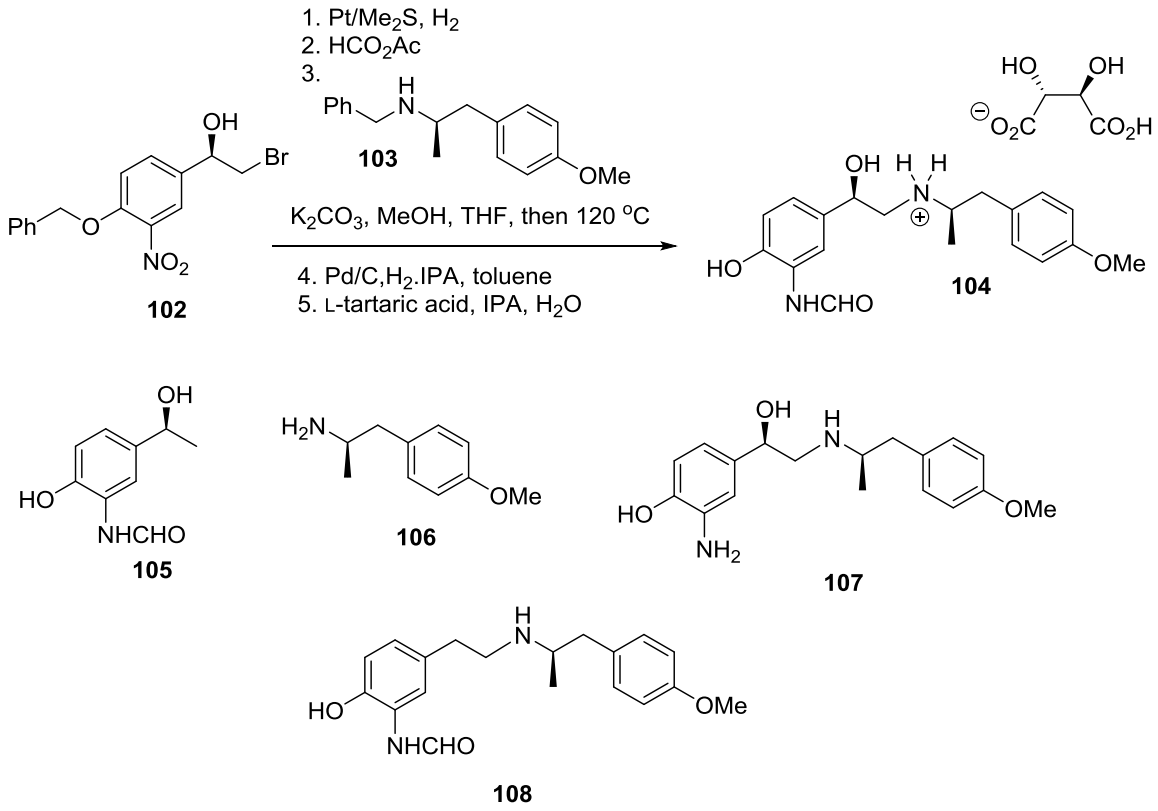
Scheme 22. Intermediate in the Synthesis of a TRVP1 Antagonist



2.7 Class I, Class II and Class III

The case of (*R*,*R*)-formoterol tartrate (**104**, Scheme 23) provides an example of the interaction between process chemistry, impurities and crystallisation outcomes. The final step in the synthesis of this compound was salt formation, which also resulted in the precipitation of a crystalline product in which compounds **105**, **106**, **107** and **108** were identified as impurities.[10, 62-64] Three crystal forms of (*R*,*R*)-formoterol tartrate **104** were identified, polymorphic forms A and B and a hydrate (form C). The initially precipitated material was form B and contained all four impurities in quantities of 0.04% to 0.64%, above the specified levels. Recrystallisation of the precipitated material from 25% aq. IPA gave crystals of form A, the thermodynamically preferred form, in which impurities **105** and **106** were no longer detectible, impurity **108** was reduced by 50%, but the levels of impurity **107** were increased due to further formamide degradation. Alternatively, warming the initially slurry of compound **104** resulted in significant re-dissolution of all four impurities, and formation of hydrate form C. Combination of re-slurrying and recrystallisation also gave low levels of all four impurities and solid material as the preferred crystal form A.[10]

Scheme 23. Route to (*R*,*R*)-Formoterol Tartrate

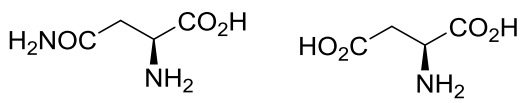


3. Incorporation of impurities within crystal lattices and faces

Section 2 presents examples of impurities occurring in process chemistry in terms of the behaviour of the impurities as they undergo crystallisation or related operations. Generally in such cases the purity or composition of batches is measured by standard sampling and analytical methods, most often by HPLC. One issue not addressed by such an approach is determination of the locations at which particular impurities are present and how the impurities can exist as components of the material at those locations. This issue has been studied, but in general separately from the management of impurities in process chemistry. However, to develop rational approaches to impurity management, better understanding of the modes of incorporation of impurities into crystalline solids is required. Hence, relevant work in that area is also examined, although most cases are not arising as a consequence of processes which would be typical of 'process chemistry'. The cases selected for this section

are 'drug-like' small organic molecules, and so the examples may be instructive when considering typically more complex API-like molecules such as those covered in Section 2.

Relatively few studies have investigated the location and supramolecular inclusion of exogenous molecules such as impurities within molecular crystals. One example concerns the crystallization of L-asparagine monohydrate (**109**, Figure 8) from water. Other amino acids present as impurities in solution can be incorporated into L-asparagine monohydrate crystals but to differing extents and distributions. Careful sequential dissolution and analysis studies on individual L-asparagine monohydrate crystals showed that most amino acid impurities were largely located on the outer or surface layers of the crystals. However, L-aspartic acid **110** was found to be incorporated into L-asparagine monohydrate crystals in significant quantities (>10%) and to be distributed relatively uniformly throughout the crystal, indicating a possible systematic substitution of L-aspartic acid molecules for L-asparagine molecules within the L-asparagine monohydrate crystals.[65] This possibility was examined by neutron diffraction studies of deuterated L-asparagine monohydrate crystals grown in the presence of deuterated aspartic acid, which showed a reduction in symmetry from $P2_12_12_1$ for L-asparagine monohydrate crystals to $P2_1$ for crystals grown with aspartic acid impurity, due to systematic substitution of aspartic acids molecules for aspargine molecules in the crystal lattice.[66]



109 **110**

Figure 8. L-Asparagine and L-Aspartic Acid

The effects of L-leucine **6** (Figure 9) impurities on the phase and morphology of L-isoleucine **5** crystals, with impurity levels ranging between 0.01 and 0.1 ratio of moles **6** to moles of **5**

has been examined. A significant reduction of crystal size was observed at 0.01-0.03 ratio but further amounts had little further effect. Impurity **6** also had an effect on the aspect ratio of the crystals. A single crystal XRD analysis was carried out on a larger crystal contaminated with **6** in ratio of 0.13, showing some distortion of the unit cell dimensions relative to that of pure **5**. It was possible to simulate substitution of molecules of **5** by molecules of **6** at lattice sites by modelling.[19]

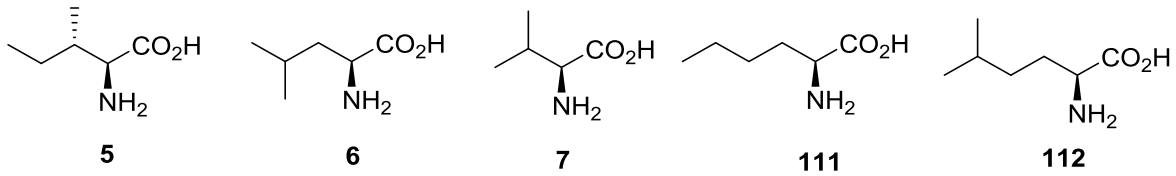


Figure 9. L-Isoleucine, L-Leucine, L-Valine, L-Norleucine and L-Homoleucine

A further study of the crystallisation of L-isoleucine **5** in the presence of related amino acids, found incorporation of L-valine **7**, L-leucine **6**, L-norleucine **111** and L-homoleucine **112** (Figure 9) in a range of 0-5% depending on amount of the impurity present in the crystallising solution. Most were in the range 0-2% with only L-leucine **6** greater than 2%. It was found the increasing incorporation of L-norleucine **111** corresponded to lengthening of the *c* axis of the unit cell of L-isoleucine **5** to accommodate the longer side chain of L-norleucine **111**.[67]

During crystallization of the phosphoantigen C-HDMAPP **113** (Figure 10) containing the impurity **114**, a significant proportion of impurity **114** remained in crystals of **113** after recrystallization. For example, crystallization of **113** from a solution containing 5.6 wt% of impurity **114** gave crystals consisting of 2.47 wt% of **114**. However, the extent of impurity **114** decreased to very low levels with slurrying of the material in water-ethanol, suggesting (with other findings) that the impure material existed as a metastable solid solution favoured

by fast crystallization kinetics. Replacement of one **113** anion per unit cell with the **114** anion was modelled and was found to create a significant free volume in the crystal packing and loss of a key H-bond in the structure, consistent with the metastability.[68]

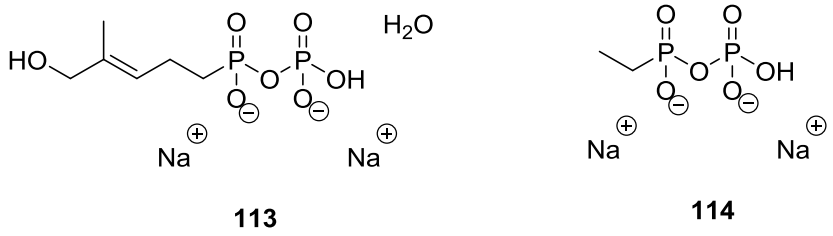


Figure 10. Phosphoantigen C-HDMAPP and Impurity

"Dutch Resolutions" involve the use of families of structurally related resolving agents, as against just one such agent in a classical resolution, providing a higher chance of success than the classical approach.[69,70] Studies on the mechanism of these resolutions suggested that a degree of incorporation of a salt of one of the resolving agents other than principal one may be involved, although kinetic effects may also be significant. For example, the related salts **115** and **116** (Figure 11) were found to form a full solid solution when simulated by modelling.[71]

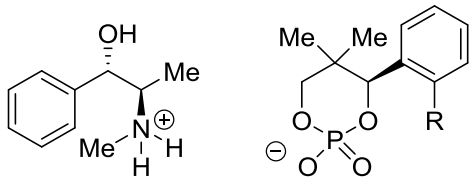
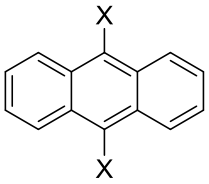


Figure 11. INAM and CLINAM

9,10-Dichloroanthracene **117** and 9,10-dibromoanthracene **118** (Figure 12) were used in a study on the crystallization and phases produced from isosteric molecules. When crystallizing

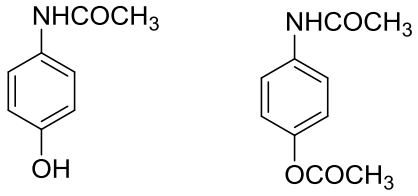
from solutions containing 1:4 to 1:6 ratios of **117** to **118**, a phase similar in lattice constants and molecular arrangement to the known structure for **118** was obtained, but having smaller unit cell volumes, consistent with incorporation of **117**. When crystallizing from solutions of **117** containing 1 mol% **118**, solid **118** was obtained, but also some thin needles which were found to be a solid solution with a very low content of **118**, ca. 3% based on occupancy refinements, in crystals of **117**.[72]



117 X = Cl
118 X = Br

Figure 12. 9,10-Dichloroanthracene and 9,10-Dibromoanthracene

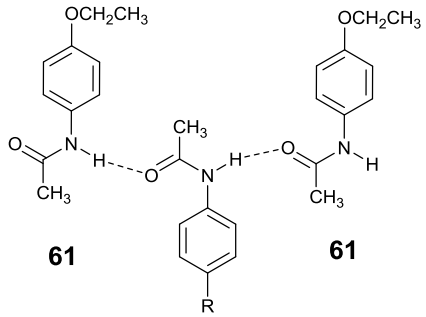
Crystallisation of acetaminophen (paracetamol) **60** by supercritical-CO_2 crystallization in the presence of p-acetoxyacetanilide **119** (Figure 13) impurity has been studied. Uptake of **119** was found to depend on the crystallization kinetics as well as the concentration of **119** in solution. The presence of impurity **119** affected the morphology of particles of **60**, giving more elongated prisms. The precipitated solid was analysed by selective dissolution, which found that **60** and **119** molecules formed a homogenous solid solution. XRD analysis indicated that incorporation of molecules of **119** into the crystal lattice of **60** caused systematic shifting of diffraction peaks to smaller angles, i.e. increased *d*-spacing. Peak spreading was found to be consistent with expansion in the *b* crystallographic direction to accommodate the longer molecules of **119** within the crystal lattice of **60**.[73]



60 **119**

Figure 13. Acetaminophen (Paracetamol) and p-Acetoxyacetanilide

In one preparation of phenacetin **61**, the starting material, 4-hydroxyacetamide (acetaminophen or paracetamol) **60**, was not completely removed after multiple recrystallizations (see Section 2.3). A stepwise dissolution study of individual phenacetin crystals found that compound **60** could be detected even in the final dissolution of the phenacetin particles. This finding could be rationalised by examination of the crystal structures of phenacetin, which contains a continuous amide hydrogen bonding chain, into which molecules of **60**, also a secondary amide, could be accommodated (Figure 14).[51] A study on the inclusion of other related secondary amides such as acetanilide **120** and 4-methoxyacetamide **121** found that these were also incorporated into the interiors of phenacetin crystals and could not be removed by washing. While concentrations of **60** and **120** were greatest near the surface of the crystals, the distribution of 4-methoxyacetanilde **121** was found to be uniform throughout the phenacetin crystals, in a proportion dependent on its concentration in solution (Figure 15 and Supplementary Information). These data show that impurities **60**, **120** and **121** can be accommodated within the interior bulk of the phenacetin crystals, significantly contributing to the difficulty of removing them. Analysis of impurity incorporation in this manner provides a direct evaluation of the challenge is removing a particular impurity by recrystallisation, as against an evaluation of the general potential for removal of an impurity that would be provided by determination of a purge factor (Section 1.2).



120 R = H
60 R = OH
121 R = OMe

Figure 14. Participation by Secondary Amide in the C4 Chain of Phenacetin

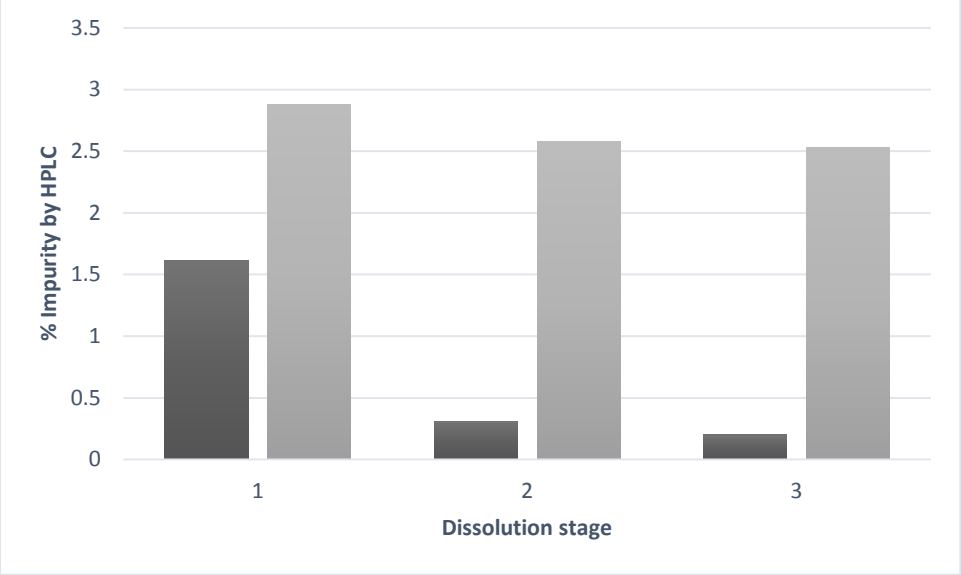


Figure 15. Relative quantities (percentage area by HPLC) of impurity compounds 120 (left column in dark grey) and 121 (right columns in light grey) incorporated into crystals of phenacetin 61 grown from solutions containing 5 mol% impurity determined at three successive dissolution stages (Supplementary Information).

Formation of partial solid solutions is observed in the crystallisation of enantiomers of malic acid, **122** and **123** (Figure 16). A detailed study of the crystal phases and corresponding phase diagrams for this system found, *inter alia*, stable and smetastable forms of the racemic

compound. These could occur as mixed crystals containing racemate and additional single enantiomer, for example, the stable racemic compound could occur with up to 70% of one enantiomer, i.e.as a partial solid solution. This observation could be rationalised on a crystal structural basis in that the racemic compound contains hydrogen bonded chains linking alternating *R* and *S* acids as 'head to head' and 'tail to tail' dimers in an alternating -*R*-*S*-*R*-*S*- sequence (Figure 17). The structure of the single *S* enantiomer likewise contains chains featuring 'head to head' and 'tail to tail' dimers, but in an –*S*-*S*-*S*-*S*- sequence (Figure 18). Partial solid solution formation is possible if, for example, an –*R*-*S*-*S*-*S*-*R*- sequence could be accommodated in the structure of the racemic compound.[74]



D-(*R*)-Malic Acid **122** L-(*S*)-Malic Acid **123**

Figure 16. Enantiomers of Malic Acid

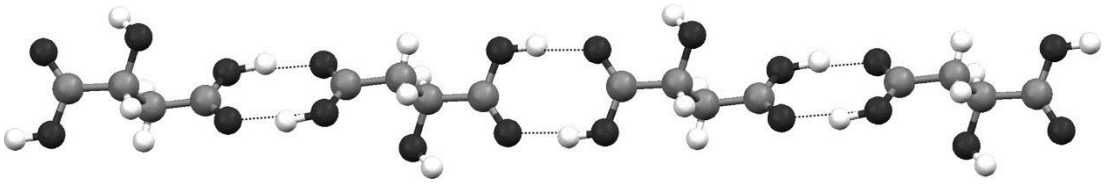


Figure 17. Molecular structure of the dicarboxylic acid chain of the stable (*R*,*S*)-malic acid (heterochiral R-S-R-S; “head-head”, “tail-tail”). Reprinted with permission from Kaemmerer, H.; Lorenz, H.; Black, S. N.; Seidel-Morgenstern, A. *Cryst. Growth Des.* 2009, *9*, 1851-1862.Copyright 2009 American Chemical Society.

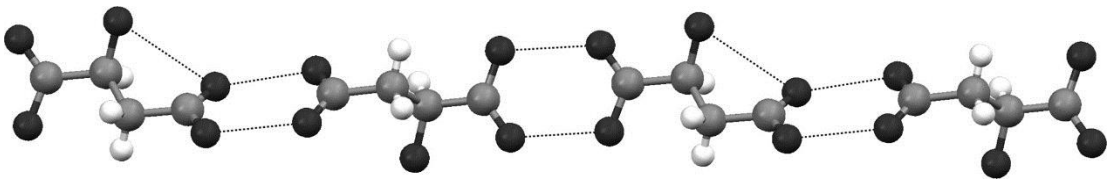
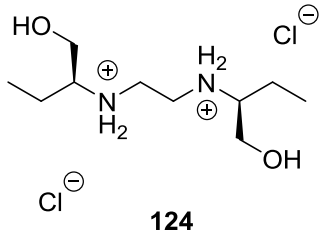


Figure 18. Molecular structure of the dicarboxylic acid chain of (*S*)-malic acid, (homochiral S−S−S−S; head-head; tail-tail). Reprinted with permission from Kaemmerer, H.; Lorenz, H.; Black, S. N.; Seidel-Morgenstern, A. *Cryst. Growth Des.* 2009, *9*, 1851-1862.Copyright 2009 American Chemical Society.

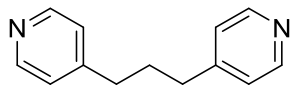
Eutectic formation provides another mode of inclusion of extraneous molecules in solid materials. For example, in the presence of fumaric or succinic acids, ethambutol dihydrochloride **124** (Figure 19) forms hygroscopic eutectics in which the microstructures of the resulting solids feature phase separated lattice domains matching those of the individual components.[75]



124

Figure 19. Ethambutol Dihydrochloride

During a study of the crystal growth mechanisms of $[Cu(bpp)_3Cl_2].2H_2O$ coordination polymer by *in situ* AFM, it was found that ligand purity was an important factor. Commercially supplied bpp ligand **125** (Figure 20) was found to contain unknown impurities in up to 5% quantities. When this material was used, AFM showed the presence of bunched steps not seen when pure ligand **125** was used (Figure 21). Impurities were strongly absorbed at terraces and steps forced to grown around these, such that the steps cannot stay straight and become bunched.[76]



125

Figure 20. Structure of Ligand 'bpp'

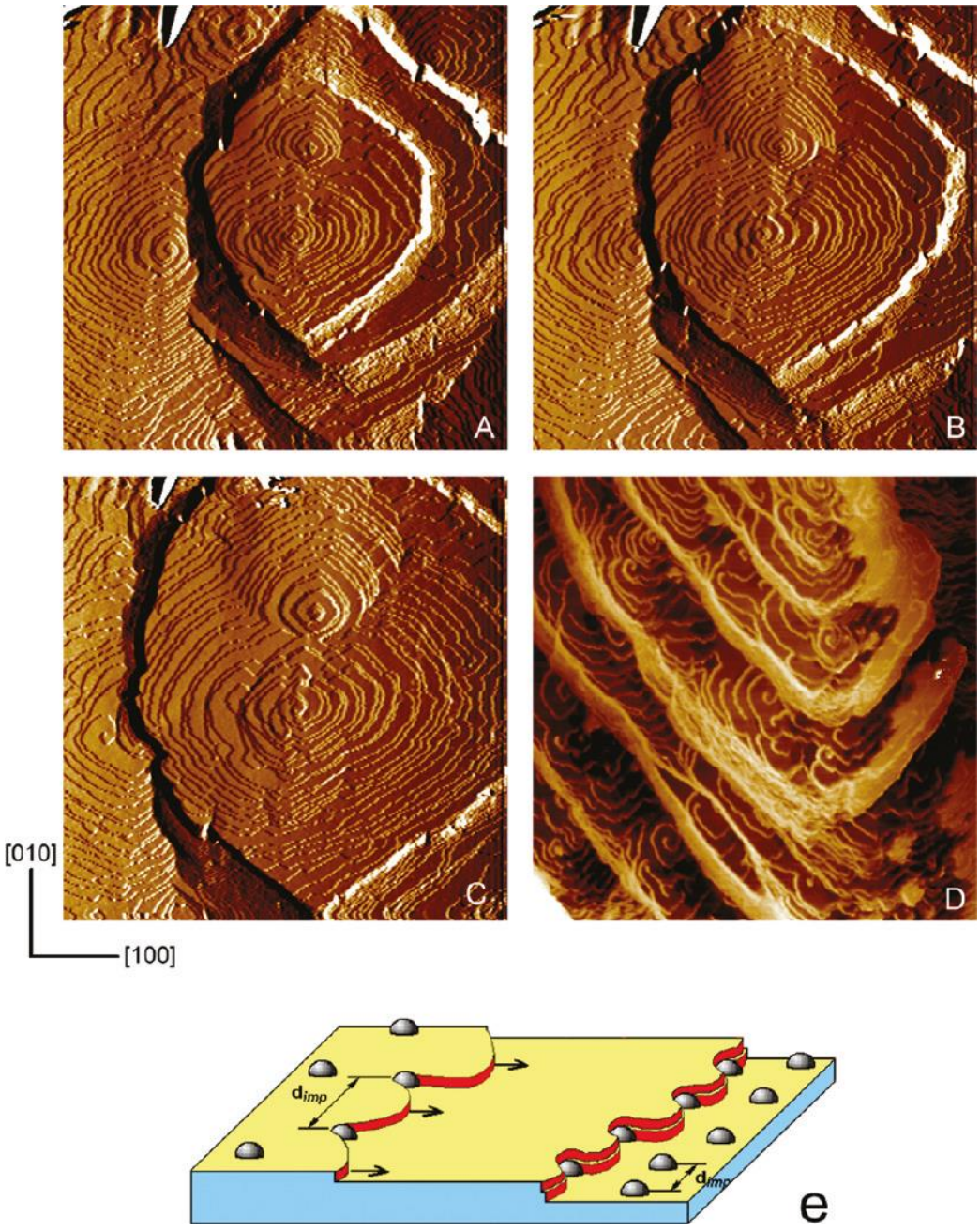


Figure 21. (A-D) AFM growth sequence (images a-c 10.9 x 10.9 μm^2, image d 15 x 15 μm^2, 28 s interval) of a (001) face in the presence of unknown impurities at supersaturation β=1.05. (e) Scheme of the Cabrera-Vermilyea model for adsorption of impurities at terraces.

Mean separation of impurities relative to their critical radius is d_{imp}. Reprinted with permission from Moret, M.; Rizzato, S. *Cryst. Growth Des.* 2009, *9*, 5035-5042. Copyright 2009 American Chemical Society.

4. Conclusions

In reviewing the behaviour and fate of impurities generated during process chemistry when undergoing crystallizations and related processes such as slurrying, a number of distinct types of behaviour were noticeable. Many impurities are satisfactorily removed by washing, recrystallization or reslurrying, i.e. Class I behaviour. However, some impurities are not adequately removed by these processes, in which case adjustment of the reaction or extraction steps may be necessary, i.e. Class 2 cases. Crystal phase transformations often provide good opportunities for impurity removal, Class 3, although the presence of impurities constitutes a challenging variable in crystal phase control.

An important question that arises in cases in which impurities are not satisfactorily removed by washing, recrystallization or reslurrying concerns the nature of the incorporation of the impurities into the crystalline material. This particular issue has not been widely studied in a process chemistry context. However, incorporation of extraneous molecules into crystal lattices has been well investigated and in many cases has been associated with formation of solid solutions. Surface inclusion of impurities has also been observed as well as eutectic formation and formation of physically separate solids.

The cases reviewed were examined for any correlation between impurity type (e.g. functional group, molecular system, reaction by which the impurity was formed) and observed behaviour. The following categories were identified as the most common discernible impurity types:

- Starting materials
- Isomers or analogues: stereoisomers, regioisomers, homologues or analogues
- Alkylation impurities, i.e. from incomplete alkylation, overalkylation or alkylation at alternate sites
- Impurities formed by dehalogenation (replacement with hydrogen) or other reduction process.
- Impurities formed by hydrolyses or the reverse
- Oxidation products, elimination products or products of other completing processes
- Palladium or other metal residues or complexes

Table 1 shows how these impurity types map to the Classes I, II and III. This is a qualitative approach and is only as representative as the cases reviewed above. The findings in Table 1 suggest that isomers and analogous compounds can have good capacity for incorporation into lattices, so may be difficult to remove by crystallisation or related processes, although in a minority of cases are quite easily removed. Residual starting materials and alkylation impurities are often removable by recrystallisation or in conjunction with a phase transformation. Dehalogenated compounds and reduced or hydrogenated impurities can have good capacity for incorporation into lattices, so may not be always easily removable but may have good potential for removal in conjunction with phase transformation. The structural similarity between any impurity and the crystallising compound will clearly be one of the factors determining its class of behaviour, however, several other factors will be significant. In particular the relative solubility of any impurity in comparison to the crystallisation compounds in the crystallisation medium is another factor which will be very significant. As solubility is strongly structure dependent, these two factors are closely linked.

Table 1. Number of instances of each category of impurities occurring in each Class of behaviour.

	Class 1	**Class 2**	**Class 3**
Starting materials and reagents	5	1	4
Isomers and analogues	6	12	0
Alkylation impurities	4	1	3
Dehalogenations or reductions	1	3	4
Hydrolyses or the reverse	3	3	4
Oxidations, eliminations or other processes	5	5	1
Metal residues or complexes	3	1	0

The issue of how and how well impurities are included in crystalline products is complex. Impurities which can reasonably replace crystallising compounds as points in the crystal lattice have the most obvious potential for incorporation, for example by forming solid solutions. Such impurities should have clear structural similarity to the crystallising compounds, be capable of fulfilling all the essential supramolecular interactions of the crystallising compounds and not possess any sterically or electronically lattice-distorting features. Several examples are given in Section 3. However, solid solutions are not the only mode of incorporation of impurities. Likely to be as important are incorporation modes which cannot be rationalised in term of crystal structures and which may be highly dependent on issues such as the degree of crystallinity, crystal mosaic spread, surface roughness, defects, solution supersaturation, the kinetics of the crystal growth process, the mode of isolation, e.g. by filtration, and the efficiency of washing of the isolated solids.

Achieving a better understanding of the inclusion of such impurities requires improved determination of the locations of impurities within crystalline samples linked with an understanding that these may be not be uniformly distributed. XRD methods can be used to detect distortions in unit cell dimension as a consequence of solid solution formation or inclusion of impurity molecules within crystal lattices. PXRD diffraction peak shifts, observation of changes in axis lengths, occupancy refinement and symmetry changes detected by neutron diffraction have been utilised for this purpose. Modelling of substitution of impurities within lattices can be used to evaluate the effect on lattice stability and the potential for inclusion. Microscopy techniques such as SEM and AFM observe changes in crystal growth phenomena arising from impurity inclusion at surfaces. Eutectic formation can be difficult to observe as XRD and spectroscopic methods are relatively insensitive toward eutectic structure. Depression of melting point determined by DSC, HSM or other thermal methods, especially when part of rigorous determination of phase diagrams, is more appropriate for the detection of eutectic formation. Sequential dissolution and analysis studies can determine occurrence of impurities at outer surface layers of crystals or distributed within crystal interiors.

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Supporting Information

The Supporting Information contains data on the incorporation into phenacetin **61** crystals of 4-phenetidine **63**, acetanilide **120** and 4-methoxyacetanilide **121**, including details of crystal growth, crystal dissolution, HPLC method, and data on relative quantities of impurity incorporation.

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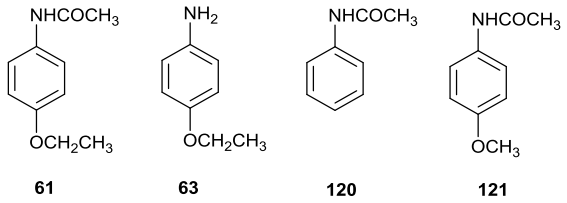
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Impurity Occurrence and Removal in Crystalline Products from Process Reactions

Humphrey A. Moynihan* and Danielle E. Horgan

SUPPLEMENTARY INFORMATION

Data on the incorporation into phenacetin 61 **crystals of 4-phenetidine** 63**, acetanilide** 120 **and 4-methoxyacetanilide** 121



61 **63** **120** **121**

Crystal growth Compounds and solvents were purchased from Sigma-Aldrich. Phenacetin (50 mg) and one of each impurity were mixed to give samples containing each impurity as either 5%, 10% or 15% of the total number of moles of each sample. These samples were dissolved in methanol (1.5 mL) with heating to 35 °C to ensure dissolution in all cases. The solutions were allow to cool and partially (but not fully) evaporated in semi-covered (partially perforated) vials to allow growth of crystals of at least 0.5 × 0.5 × 0.2 mm dimensions. The crystals were isolated, washed with 0.4 mL of cold methanol, dried under vacuum and weighed.

Crystal dissolution For each impurity and concentration, individual crystals were subjected to a series of three partial dissolutions followed by analysis of the resulting solutions at each stage. The mass of the crystals determined the volumes of solvent used for complete dissolution, to give a concentration of 1.0 mg/mL, e.g. 6.0 mL was used for the complete dissolution of a crystal weighing 6.0 mg, with. 2.0 mL used for each of the three partial dissolution stages. At the beginning of the series of dissolutions, the crystal was placed in a sample vial. The correct volume of solvent was added and the crystal was observed partially dissolving. It was then removed and washed with a small quantity of cold hexane before being placed in a second sample vial to be further dissolved. This process was repeated for a third final stage giving complete dissolution. The solutions remaining in the sample vials were analysed by HPLC. Each of these experiments was repeated three times.

HPLC HPLC analysis on the products was conducted on an Agilent 1200 series HPLC, with a YMC-Pack ODSA column (250 x 4.6 mm, 5 μm). An isocratic 60:40 MeOH:H_2O solvent system was employed, at a flow rate of 1 mL/min and an injection volume of 10 μL. The detector was set at 254 nm and the oven temperature was ambient.

Table S1 Relative quantities (percentage area by HPLC) of impurity compounds **63**, **120** and **121** incorporated into crystals of phenacetin **61** determined at three successive dissolution stages.

Impurity	Mol % impurity in solution	% impurity in 1st dissolution layer	% impurity in 2nd dissolution layer	% impurity in 3rd dissolution layer
63	5, 10 or 15	<LOQ[a]	<LOQ[a]	<LOQ[a]
120	5	1.61 ± 0.01	0.31 ± 0.04	0.20 ± 0.01
120	10	2.28 ± 0.02	0.56 ± 0.02	0.45 ± 0.01
120	15	5.89 ± 0.03	1.44 ± 0.02	0.49 ± 0.03
121	5	2.88 ± 0.02	2.58 ± 0.01	2.53 ± 0.02
121	10	6.11 ± 0.01	5.23 ± 0.02	4.97 ± 0.01
121	15	7.64 ± 0.02	7.36 ± 0.04	6.52 ± 0.03

a 3.5×10^{-3} mg/mL