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Use of ω-Transaminase Enzyme Chemistry in the Synthesis of a JAK2 Kinase Inhibitor

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ABSTRACT: ω-Transaminase enzyme chemistry provides an excellent methodology to build synthetically useful chiral amines from their corresponding ketones. An application of this methodology, providing a long-term commercial manufacturing route to a JAK2 kinase inhibitor, is reported herein.

INTRODUCTION

The API **1** is an orally active inhibitor of the JAK2 kinase[1,2] with the potential to be a curative treatment for idiopathic myelofibrosis (IMF) and polycythaemia rubra vera (PRV). The compound was discovered by AstraZeneca, at its Boston research site in North America, and was subsequently developed at the Macclesfield facility in the UK.

The Medicinal Chemistry route (shown in Scheme 1) was used for the production of the first manufacturing batch (400 g) in the large-scale laboratory at Macclesfield to supply the 1 month toxicological study. Very limited development work was possible in the short timeframes available during preclinical development, and the route was scaled with minimal improvement work.

Production of the coupling partner **7** was relatively trivial: yields of 90% were achieved from commercially available 3-amino-5-methylpyrazole and 2,4,5-trichloropyrimidine. The process was noteworthy only for the excellent selectivity for displacement at the 4-position of the pyrimidine and was subsequently scaled to 23 kg (input) with only minor changes. The main part of the synthetic route (Scheme 1), however, presented a number of challenging problems, not the least of which was an overall yield to the API of only 2% from 2,4-dichloro-5-fluoropyrimidine (**2**).

Water was difficult to remove during the workup and contaminated the product of the dechlorination reaction leading to 2-chloro-5-fluoropyrimidine (**3**). The next stage was sensitive to water, so that **3** had to be reworked twice to furnish suitable quality material.

Cyanation of **3** was initially achieved with an all-in process at 100 °C but was subject to a large exotherm (166 kJ/mol) (with initiation at >100 °C). Large amounts of tar formation were observed that, in combination with the metal residues, made workup extremely difficult even on relatively small scale. The reaction was also capricious in that it was prone to stalling before completion and, once stopped, could not be restarted through the addition of more reagents or catalyst. Filtration through a silica plug and a rework were necessary to produce **4** of suitable quality.

Tar formation also hindered the reaction to form **5**, which was low yielding, operationally difficult, and in need of full chromatography and an additional rework. Despite excellent enantioselectivity, the Rh-DuPhos catalyst used for the reduction to produce the chiral amine **6** was expensive, and the extremely water-soluble amine required BOC protection to enable extraction into an organic solvent. The BOC group was subsequently removed under anhydrous conditions. Coupling of **6** and **7** (to form **1**) was prone to significant and unpredictable levels of epimerization (60−90% ee), such that chiral chromatography was necessary to furnish the chirally pure API **1**.

RESULTS AND DISCUSSION

A further, larger campaign was run almost simultaneously with the target of producing 5 kg of API for phase I clinical trials and formulation. A commercial source of 2-chloro-5-fluoropyrimidine (**3**) was found, and outsourcing of the subsequent two stages furnished vinylacetamide **5**, with modest improvements in processing and yield. We introduced distillation of cyanopyrimidine **4**, leading to reduced tar levels in the subsequent vinylacetamide **5** stage. Reduction to the chiral amine **6** was scaled with little further work. Development of the coupling step, however, was far from straightforward. The cause of the epimerization during the reaction remained unknown, and chiral chromatography was undesirable at the larger scale. From our investigations, it became clear that, whilst the product of the reaction itself appeared to be stable under the reaction conditions, it was in fact the chiral amine **6** that was epimerising as the reaction progressed, such that the % ee of API **1** slowly decreased as the reaction progressed due to coupling of the unwanted enantiomer of **6** (Figure 1).

Some potential mechanisms (imine formation, radical mechanism) were proposed and ruled out through experimentation. Computer modelling proved very useful to rule out deprotonation by triethylamine followed by nonselective reprotonation (confirmed also by experimentation: triethylamine alone cannot racemise the amine). A more likely mechanism, proposed by the modelling studies, proceeds via an enamine or an ylide obtained from the action of triethylamine

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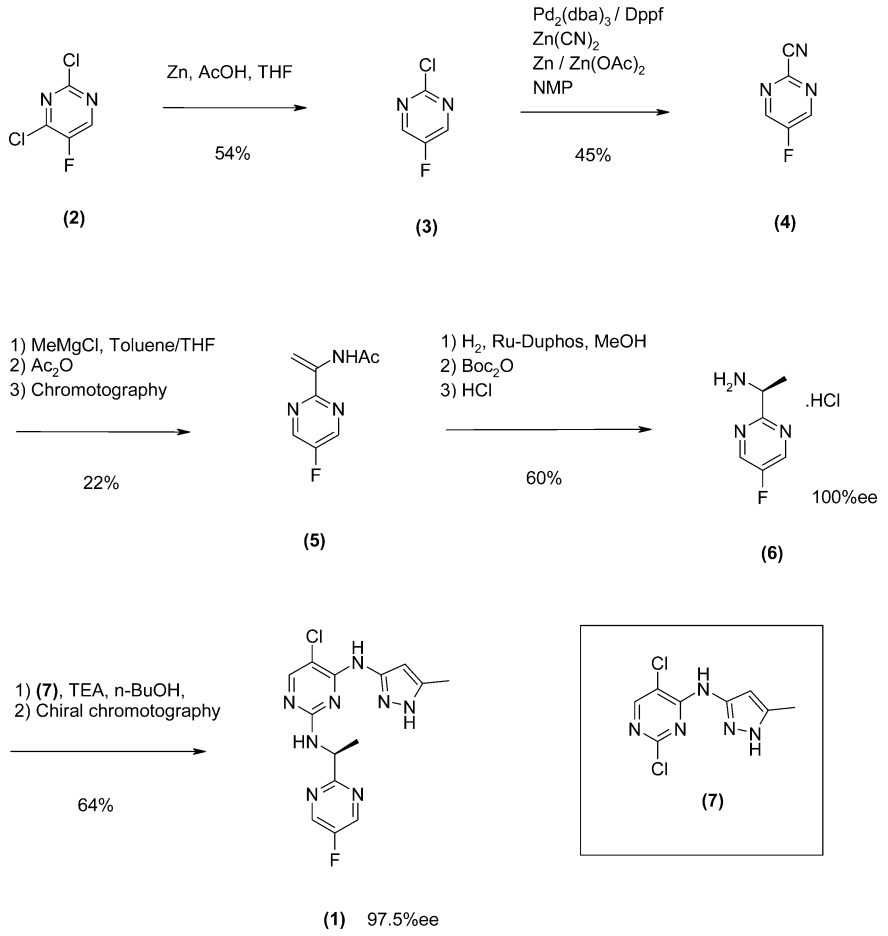
Scheme 1. Medicinal Chemistry route



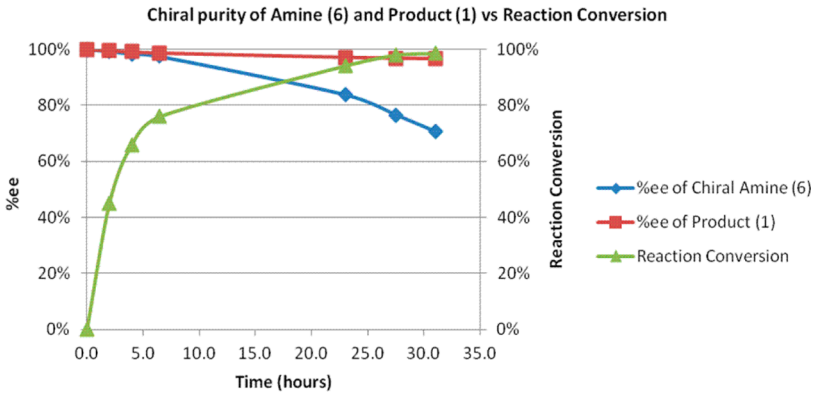


Figure 1. Chiral purity of amine (**6**) and product (**1**) during the reaction (using $KHCO_3$).

and triethylammonium ion present in the buffered system (Scheme 2). It was predicted that the epimerization should be greatly reduced in the absence of such a buffer system. This might be achieved through the use of a different base whose conjugate acid would not remain in solution.

Changing the base from triethylamine to potassium bicarbonate meant that the HCl was removed from the solution (by precipitation of KCl). This led to a significant reduction of the extent of epimerization, producing **1** of >94% ee in the crude reaction mixture. Crystallisation as the maleate salt (eutectic point at 92% ee) allowed chiral purification without the need for chromatography and also gave excellent processing properties (Scheme 3).

API **1** was crystallised (after neutralisation and extraction into isopropyl acetate) by slow antisolvent addition of *n*-heptane. Oiling prior to crystallisation was a major problem, and the kinetics of the crystallisation were seen to be unusually slow (additions of >24 h were necessary to prevent oiling). Redesign of this final isolation would be required for robust larger-scale manufacture, but it proved acceptable for the early deliveries. With the improvements made to the process, the second manufacturing campaign produced 7 kg of chirally pure material although the overall yield from commercially available starting material **3** was still only approximately 4%.

Recognising the difficulty of the synthesis to the chiral amine **6**, further work was performed to try to optimise the existing

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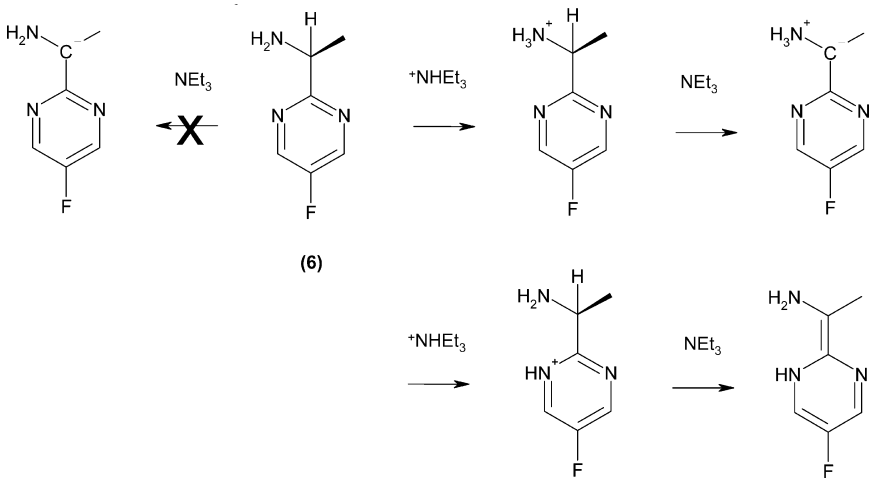
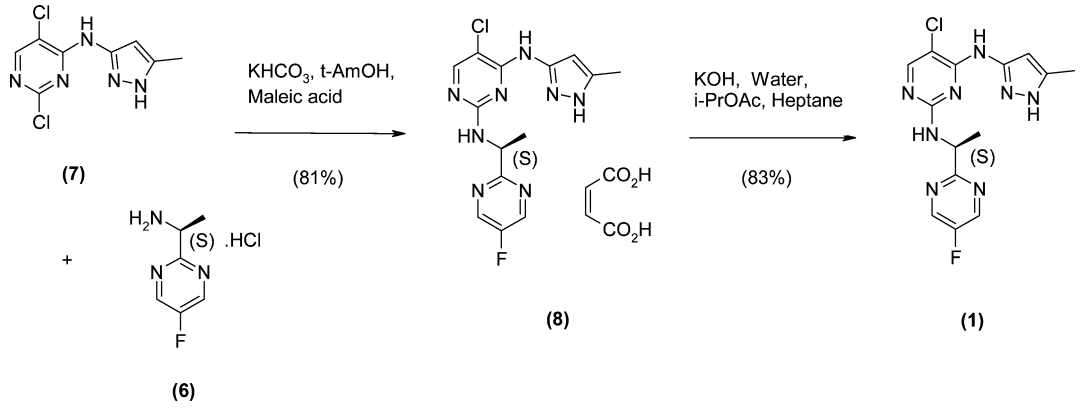
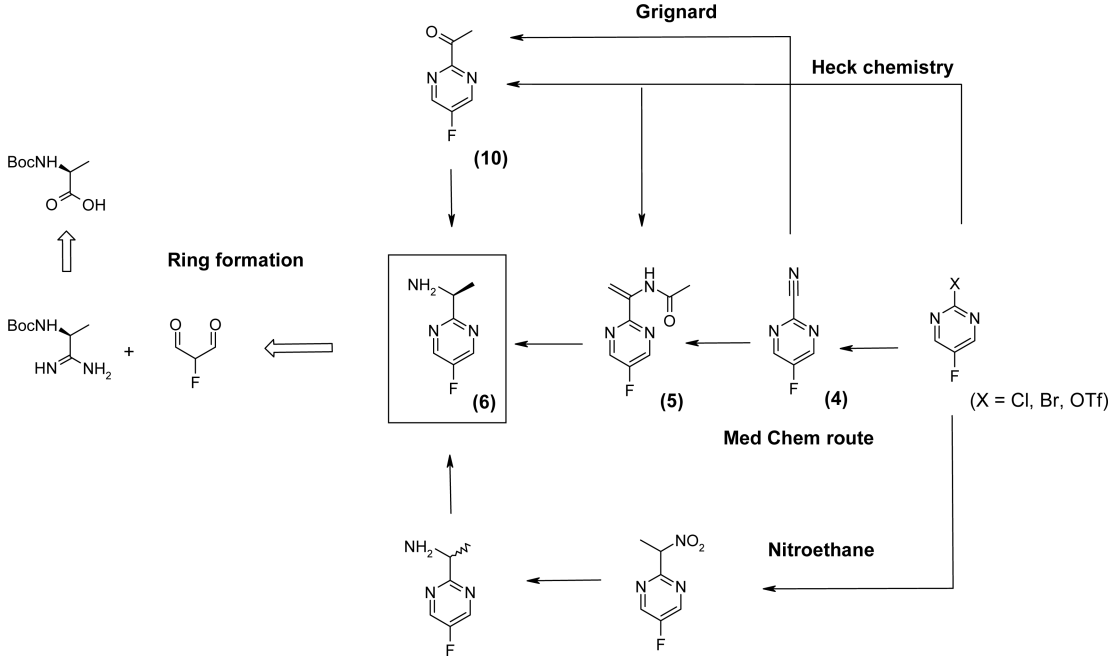
Scheme 2. Postulated ylide and enamine mechanisms



Scheme 3. Coupling and purification stages



Scheme 4. Alternative approaches to the chiral amine (6)



low-yielding, operationally difficult vinylacetamide **5** step of the medicinal chemistry route. A study utilising process analytical technology (PAT) (UV−vis and mid-IR) was undertaken which showed clean formation of the intermediate imine from

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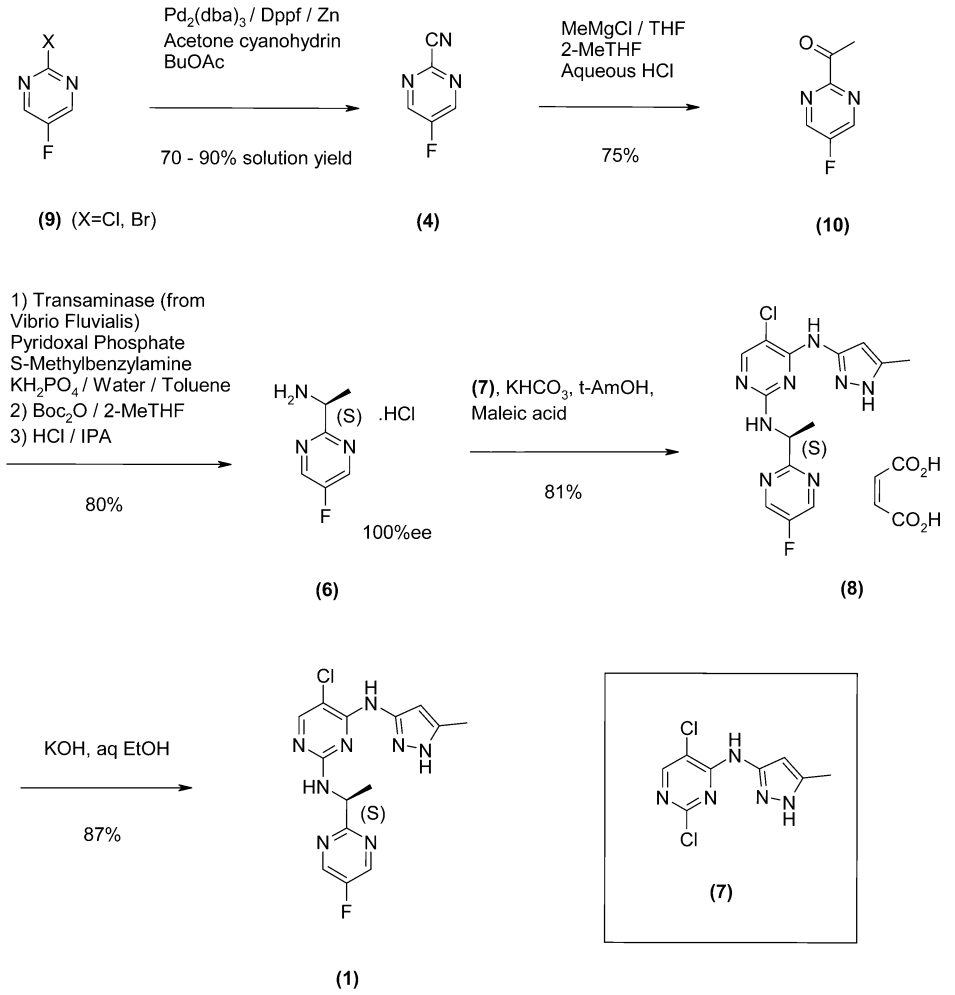
Scheme 5. Proposed long-term manufacturing route



cyanopyrimidine **4** and corresponding high solution yield (87%) of the ketone **10** formed by acidic quench. Problems in the process were shown to occur during conversion of the imine to vinylacetamide **5** by reaction with acetic anhydride, although it was difficult to discern the nature of any nonproductive pathways. Attempts to improve the solution yield of vinylacetamide **5** by varying the mode and temperature of addition were unsuccessful and offered no improvement over the process used previously for manufacture (48% solution yield). The purity of **5** was also not improved by any of the process modifications tested which precluded isolation by crystallisation without the need for chromatography.

Seemingly fundamental problems with the synthetic route therefore provoked an investigation into alternative routes to chiral amine **6** (Scheme 4).

Attempts using such methodologies as Heck chemistry (utilising vinyl butyl ether and hydrolysis)[3] to give the ketone **10**, palladium-catalysed insertion of nitroethane,[4] and building the pyrimidine ring, all suffered from low yields and required extensive chromatography. The only seemingly viable alternative to emerge from this work centred on the Grignard addition to the cyanopyrimidine **4** to deliver ketone **10**, and ultimately it was from this approach that the proposed long-term manufacturing route was developed (Scheme 5).

Work to improve the operational difficulties of the existing cyanation chemistry proved fruitful. A switch to acetone cyanohydrin as cyanide source[5] and BuOAc as solvent gave clean conversion of 2-chloro-5-fluoropyrimidine to product with controllable exotherm through the slow addition of the (liquid) acetone cyanohydrin. A 70% solution yield was obtained in the laboratory, (although a complete workup procedure was not realised before the project was terminated, and the chemistry was never scaled beyond the laboratory). Higher solution yields (of 90%) were achieved using 2-bromo-5-fluoropyrimidine.

Clean conversion of the cyanopyrimidine **4** to the ketone **10** was realised with relatively straightforward processing, via conventional Grignard chemistry. This reaction was demonstrated on 20 L scale with little problem.

With a promising route to the ketone in hand, numerous strategies were explored to convert **10** to chiral amine **6** (see Scheme 6).

Many of these processes exposed the inherent fragility of the pyrimidine ring, and the formation of oily tars was commonplace. The only promising alternative identified was an enzymatic approach using transaminases, the conditions of which were by necessity very mild and provided the added attraction of a single chemical processing step.

Enzymatic transamination reactions are important and abundant in nature and involve the transfer of an amino group from α-amino acids to α-keto acids. Despite this, examples of industrial applications[6] for the transfer of an amino

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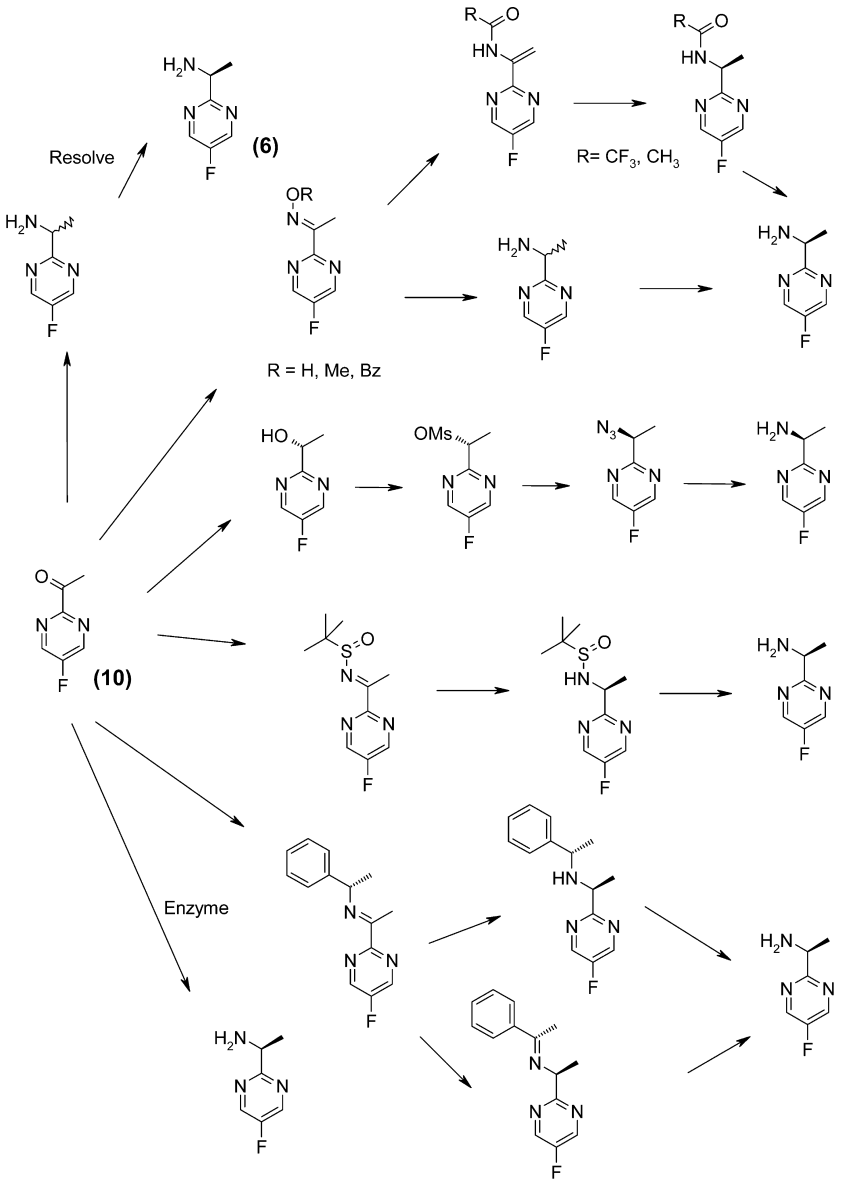
Scheme 6. Approaches for the conversion of ketone (10) to the chiral amine (6)



group (from a non-α-amino acid amine donor) to simple ketones are not common within the literature. However, in recent years some notable examples have been described such as the preparation of sitagliptin by Merck.[7]

An initial screen with ketone **10** highlighted almost complete and clean conversion with excellent chiral selectivity using *Vibrio fluvialis* enzyme and (*S*)-α-methylbenzylamine as the amine donor, with pyridoxal phosphate cofactor in an aqueous buffer. This clearly presented an opportunity to utilise this methodology; a detailed discussion of the development of this reaction step is included in a related publication,[8] whilst an overview is presented herein. Enzyme inhibition was observed at higher concentrations of acetophenone byproduct, leading to the use of high dilution (100 volumes). Under these conditions, the reaction required greater than equimolar concentrations of the (*S*)-α-methylbenzylamine (relative to the ketone **10**), which could not be separated from the product by the downstream processing.

These issues were neatly solved (utilising Kim et al. methodology[9]) by the use of an organic cosolvent (toluene), which extracted the acetophenone from the aqueous phase as it was formed (see Scheme 7). This had the dual advantage of avoiding enzyme inhibition and also driving the equilibrium reaction such that only a small (and tolerable) excess of the (*S*)-α-methylbenzylamine (1.1 equiv) was necessary to push the reaction to completion. Scaling up to 20 L scale produced 500 g of good quality amine **6**. The relatively high loading of formulated *V. fluvialis* enzyme (2.8 mL per 1 g ketone **10**) did lead to extended filtration times during processing, and subsequent work sought to identify an enzyme that could be used with a much lower loading. Forty-seven commercially available transaminase enzymes were screened for activity, and the Codexis enzyme, TA-P1-A06, was subsequently selected for evaluation. This enzyme was successfully used to prepare amine **6** in 68% yield from ketone **10** with >99.5% ee with a loading of 0.025 g/g ketone **10**.

No further development work was performed on the coupling stage (to produce **8**) though the process was successfully proven on 100 L scale. The purification process to produce API **1** was subsequently redesigned such that

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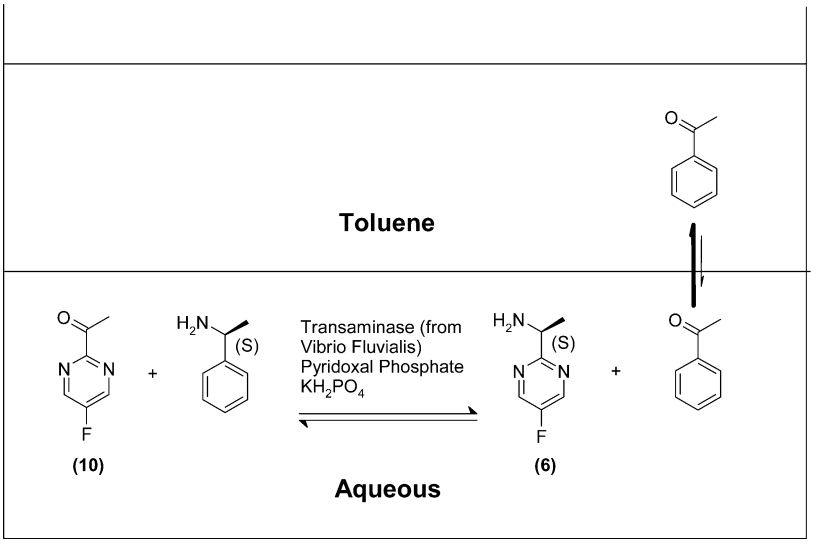
Scheme 7. Equilibrium of enzyme reaction



addition of KOH to a hot aqueous EtOH solution of maleate salt **8** followed by cooling gave an excellent crystallisation, without the oiling that plagued the isopropyl acetate/*n*-heptane method, or the need for extraction. This procedure was demonstrated on 100 L scale in the large scale laboratory.

The project was terminated in 2012, and whilst considered viable as a long-term manufacturing route, the final route was not scaled further. Laboratory yields of API suggest that an overall yield from commercially available material could be >30%.

CONCLUSION

We report a manufacturing route to the API **1**, utilising ω-transaminase enzyme chemistry to construct the key chiral amine coupling partner. Examples of this methodology, though known in the literature, are limited to mostly lab-scale procedures, and few examples[6,7] exist of their application on scale. Development work of this enzymatic stage, utilising an elegant two-phase reaction mixture to avoid inhibition of the enzyme (and therefore concentrate the process), has made this a commercially viable step. This paper demonstrates a useful addition to the application of ω-transaminase enzyme methodology.

EXPERIMENTAL SECTION

Preparation of 2,5-Dichloro-*N*-(5-methyl-1*H*-pyrazol-3-yl)pyrimidin-4-amine (7). 3-Amino-5-methylpyrazole (12.9 kg, 1.05 mol equiv) was charged to a vessel and dissolved in IMS (67.3L, 2.9 rel vols) to give a yellow solution. Meanwhile, 2,4,5-trichloropyrimidine (23.2 kg, 1.0 mol equiv), IMS (67.3 L, 2.9 rel vols) and triethylamine (16.6 kg, 1.3 mol equiv) were charged to a second vessel and agitated. The 3-amino-5-methylpyrazole solution was then added slowly at 20−25 °C, and the mixture was left stirring at 20−25 °C for 20 h. Water (227.4 L, 9.8 rel vols) was slowly added and the stirred mixture held for 1 h before being filtered under vacuum. The solid was then washed with water (78.9 L, 3.4 rel vols) before being placed in a vacuum oven at 40 °C to dry. The product was isolated as a white crystalline solid (27.9 kg, 90% yield). ^{1}H NMR (400 MHz, DMSO) δ: 2.26 (s, 3H), 6.29 (s, 1H), 8.32 (s, 1H), 9.70 (s, 1H), 12.31 (s, 1H). ^{13}C NMR (100 MHz, DMSO) δ: 157.51 (s), 157.23 (s), 155.76 (s), 145.87 (s), 139.54 (s), 113.64 (s), 98.61 (s), 11.30 (s). HRMS Calcd for $C_8H_8Cl_2N_5$: 244.0151; HRMS found [M + H]+: 244.0148.

Preparation of 5-Fluoropyrimidine-2-carbonitrile (4). Charged were 1,1′-bis(diphenylphosphino)ferrocene)-palladium(II) chloride (303 mg, 0.01 mol equiv), 2-chloro-5-fluoropyrimidine (7.0 g 1.00 mol equiv), triethanolamine (5.56 mL, 1.1 mol equiv), butyl acetate (19.74 mL 3.0 rel vols), and zinc dust (292 mg, 0.12 mol equiv) to the reaction vessel. The mixture was degassed and inerted thoroughly with nitrogen. It was heated to 105 °C, and acetone cyanohydrin (3.57 mL, 1.10 mol equiv) was charged at 105 °C over 2 h via syringe pump. Then the mixture was cooled to ambient temperature and filtered; washing with butyl acetate yields an orange solution (48 mL, 70% yield based on solution assay) (90% solution yield can be achieved using 2-bromo-5-fluoropyrimidine as input). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.17 (d, 2H, J = 0.88 Hz). ^{13}C NMR (100 MHz, DMSO) δ: 157.10 (d, J = 270.9 Hz), 147.51 (d, J = 22.6 Hz), 139.60 (d, J = 6.0 Hz), 115.57 (s). GC/MS: m/z 123 M^+ (and fragments consistent with the proposed structure).

Preparation of 1-(5-Fluoropyrimidin-2-yl)ethanone (10). A solution of 2 M MeMgCl (268 mL, 0.81 mol) in tetrahydrofuran was added to a solution of 5-fluoropyrimidine-2-carbonitrile (**4**) (82.5 g, 0.65 mol) in 2-methyltetrahydrofuran (600 mL) at −40 °C. On complete reaction, the reaction mixture was warmed to −25 °C and transferred into a solution of aqueous hydrochloric acid (475 mL, 1.98 mol). On complete reaction, the phases were separated, and the aqueous phase was extracted further with 2-methyltetrahydrofuran (300 mL). The organic phases were combined and concentrated by evaporation before *n*-heptane was added to crystallize the product as a light-brown crystalline solid (73.2 g, 80%). ^{1}H NMR (400 MHz, DMSO) δ: 9.09 (d, 2H, J = 0.86 Hz), 2.68 (s, 3H). ^{13}C NMR (100 MHz, DMSO) δ: 195.81 (s), 157.15 (d, J = 269.2 Hz), 156.73 (d, J = 5.6 Hz), 146.18 (d, J = 21.1 Hz), 27.59 (s). ^{19}F NMR (367.5 MHz, $CDCl_3$) δ: −132.2 (s).

Preparation of (1*S*)-1-(5-Fluoropyrimidin-2-yl)-ethanamine (6) on 20 L scale. (*S*)-α-methylbenzylamine (450 mL, 1.10 mol equiv) was added to a solution of monobasic potassium phosphate (87.4 g, 0.20 mol equiv) in water (6.75 L, 15.0 rel vols). The pH of the solution was

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adjusted to pH 7.5 by the addition of acetic acid (variable amount). Pyridoxal phosphate (4.3 g, 0.005 mol equiv) was added, followed by 1-(5-fluoropyrimidin-2-yl)ethanone (**10**) (450 g, 1.0 mol equiv), a buffered solution of *V. fluvialis* transaminase enzyme (174.6 KU), and toluene (2.25 L, 5.0 rel vols). The reaction mixture was adjusted to pH 7.5 with potassium carbonate (variable amount) and then held at 29 °C for 18 h. The reaction was filtered and the organic layer discarded. Potassium carbonate (887.7 g, 2.0 mol equiv) was added to the aqueous phase followed by a solution of di-*tert*-butyl dicarbonate (771.0 g, 1.1 mol equiv) in 2-methyltetrahydrofuran (3.6 L, 8.0 rel vols). The mixture was filtered and the aqueous layer extracted with further 2-methyltetrahydrofuran (1.8 L, 4.0 rel vols). The organic layers were combined and concentrated at atmospheric pressure to a contents temperature of 98 °C. 5−6N hydrochloric acid in isopropanol (1.5 L, 2.7 mol equiv) was added at ambient temperature. The reaction mixture was heated to 40 °C to precipitate the product, which was isolated as a crystalline solid (451 g, 79%). ^{1}H NMR (400 MHz, DMSO) δ: 9.03 (d, 2H, J = 1.08 Hz), 4.54 (q, 1H, J = 6.90 Hz), 1.60 (d, 3H, J = 6.90 Hz). ^{13}C NMR (100 MHz, DMSO) δ: 162.57 (d, J = 5.2 Hz), 157.15 (d, J = 269.2 Hz), 145.52 (d, J = 20.7 Hz), 50.72 (s), 18.81 (s). ^{19}F NMR (367.5 MHz, DMSO) δ: −138.7 (s). HRMS Calcd for $C_6H_9FN_3$: 142.0775; HRMS found [M + H]+: 142.0774. Enantiomeric excess was determined by chiral HPLC (CrownPak CR+, aqueous perchloric acid, >99% ee).

Preparation of (1*S*)-1-(5-Fluoropyrimidin-2-yl)-ethanamine (6) using Codexis TA-P1-A06. (*S*)-α-Methylbenzylamine (18.9 mL, 0.15 mol) was added to a solution of monobasic potassium phosphate (3.6 g, 0.27 mol) in water (300 mL). The pH of the solution was adjusted to pH 7.5 by the addition of acetic acid (7.0 mL, 0.12 mol). Pyridoxal phosphate (0.16 g, 0.0007 mol) was added, followed by 1-(5-fluoropyrimidin-2-yl)ethanone (**10**) (20.0 g, 0.13 mol), TA-P1-A06 enzyme (0.47 g), and toluene (100 mL). The reaction mixture was adjusted to pH 7.5 with potassium carbonate (1.84 g, 0.013 mol) and then held at 29 °C for 18 h. Celite (4.0 g) was added, and the reaction mixture was filtered. A mixture of toluene (40 mL) and water (100 mL) was added to the reaction vessel, and the reaction mixture was stirred and discharged as a wash to the filter bed. The aqueous phase was separated and the organic phase discarded. Potassium carbonate (36.8 g, 0.27 mol) was added to the aqueous phase followed by a solution of di-*tert*-butyl dicarbonate (31.9 g, 0.15 mol) in 2-methyltetrahydrofuran (160 mL). The mixture was heated to 40 °C for 18 h and the resulting biphasic mixture filtered. The organic layer was separated and evaporated to dryness. The residue was dissolved in MTBE (58 mL) and a solution of 5−6N hydrochloric acid in isopropanol (72.0 mL, 0.36 mol) was added. The reaction mixture was heated to 40 °C for 24 h. An off-white suspension was formed. The reaction mixture was cooled to 25 °C and heptane (60 mL) was added. After 1h the reaction mixture was filtered. The isolated solid was washed with 2-methyltetrahydrofuran (40 mL) and dried in vacuo to give the amine **6** as a monohydrochloride salt (16.1 g, 68%). ^{1}H NMR (400 MHz) δ: 9.02 (d, 2H), 4.55 (m, 1H), 1.58 (d, 3H). HRMS Calcd for $C_6H_9FN_3$: 142.0775; HRMS found [M + H]+: 142.0774. Enantiomeric excess was determined by chiral HPLC (Chiralpak AD3 5 cm × 0.46 cm, >99.5% ee).

Preparation of 5-Chloro-*N*^{2}-[(1*S*)-1-(5-fluoropyrimidin-2-yl)ethyl]-*N*^{4}-(5-methyl-1*H*-pyrazol-3-yl)-pyrimidine-2,4-diamine; Maleic Acid (8). 2,5-Dichloro-*N*-(5-methyl-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**7**) (100 g, 1.0 mol equiv), (1*S*)-1-(5-fluoropyrimidin-2-yl)ethanamine (**6**) (90.96 g, 1.25 mol equiv), and potassium bicarbonate (164.2 g, 4.0 mol equiv) were stirred in *tert*-amyl alcohol (331 mL, 4.1 rel vols) at 100 °C for 50 h. The resulting slurry was cooled to 50 °C and washed twice with water (400 mL, 4.0 rel vols) at 50 °C. Isopropanol (800 mL, 8.0 rel vols) was added before a solution of maleic acid (47.6 g, 1.0 mol equiv) in isopropanol (400 mL, 4.0 rel vols) is charged. Further isopropanol (200 mL, 2.0 rel vols) was added before cooling to 0 °C, filtering, and washing with MTBE (300 mL, 3.0 rel vols). The product was dried at 50 °C and isolated as a white solid (153 g, 81% yield). ^{1}H NMR (500 MHz, DMSO) δ: 1.54 (d, 3H, J = 7.0 Hz), 2.22 (s, 3H), 5.25 (m, 1H), 6.21 (s, 2H), 7.93 (s, 1H), 8.77 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.3, (s), 166.4 (s), 158.6 (s), 157.7 (d, J = 260.1 Hz), 154.8 (s), 152.4 (s), 145.0 (d, J = 20.7 Hz), 144.7 (s), 140.5 (s), 130.9 (s), 102.4 (s), 96.1 (s), 52.5 (s), 20.6 (s), 11.4 (s). ^{19}F NMR (367.5 MHz, $CDCl_3$) δ: −140.9 (s). HRMS Calcd for $C_{14}H_{15}ClN_8F$: 349.1092; HRMS found [M + H]+: 349.1080.

5-Chloro-*N*^{2}-[(1*S*)-1-(5-fluoropyrimidin-2-yl)ethyl]-*N*^{4}-(5-methyl-1*H*-pyrazol-3-yl)pyrimidine-2,4-diamine (1). 5-Chloro-N2-[(1*S*)-1-(5-fluoropyrimidin-2-yl)ethyl]-N4-(5-methyl-1*H*-pyrazol-3-yl)pyrimidine-2,4-diamine maleic acid (**8**) (130.0 g, 1.0 mol equiv) was charged to a vessel with ethanol (494 mL, 3.8 rel vols), water (611 mL, 4.7 rel vols), and 49% aqueous potassium hydroxide (45.88 g, 1.5 mol equiv). The mixture was heated to 60 °C to dissolve the substrate, screened through an in-line filter, and washed with 50% aqueous ethanol (195 mL, 1.5 rel vols). The solution was then seeded with **1** (0.975 g, 0.0075 rel wt) and then cooled to crystallise the product. A further portion of water (650 mL, 5.0 rel vol) was added to increase the recovery. The product was then filtered and washed with a mixture of water (260 mL, 2.0 rel vol) and ethanol (130 mL, 1.0 rel vol) before drying at 40 °C to constant weight. The product was isolated as a white crystalline solid (81.1 g, 87% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.61 (d, 3H, J = 7.03 Hz), 2.32 (s, 3H), 5.39 (q, 1H, J = 7.03 Hz), 7.61 (s, 1H), 7.94 (s, 1H), 8.59 (s, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 167.4 (d, J = 6.0 Hz), 159.6 (s), 156.6 (d, J = 263.9 Hz), 154.5 (s), 145.0 (s), 144.5 (s), 140.2 (s), 103.3 (s), 94.8 (s), 52.9 (s), 21.1 (s), 12.4 (s). ^{19}F NMR (471 MHz, $CDCl_3$) δ: −140.1 (s). HRMS Calcd for $C_{14}H_{15}ClN_8F$: 349.1092; HRMS found [M + H]+: 349.1093.

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Notes

The authors declare no competing financial interest.

REFERENCES

(1) Deshpande, A.; Reddy, M. M.; Schade, G. O. M.; Ray, A.; Chowdary, T. K.; Griffin, J. D.; Sattler, M. *Leukemia* **2012**, *26* (4), 708−715.

(2) Ioannidis, S.; Lamb, M. L.; Wang, T.; Almeida, L.; Block, M. H.; Davies, A. M.; Peng, B.; Su, M.; Zhang, H.-J.; Hoffmann, E. *J. Med. Chem.* **2011**, *54* (1), 262−276.

(3) Liu, S.; Berry, N.; Thomson, N.; Pettman, A.; Hyder, Z.; Mo, J.; Xiao, J. *J. Org. Chem.* **2006**, *71* (19), 7467−7470.

(4) Vogl, E. M.; Buchwald, S. L. *J. Org. Chem.* **2002**, *67* (1), 106−111.

(5) Sundermeier, M.; Zapf, A.; Beller, M. *Angew. Chem., Int. Ed.* **2003**, *42* (14), 1661−1664.
(6) Liese, A.; Seelbach, K.; Wandrey, C. In *Industrial Biotransformations*, 2nd ed.; Wiley-VCH: Weinheim, 2006.
(7) Savile, C. K.; Janey, J. M.; Mundorff, E. C.; Moore, J. C.; Tam, S.; Jarvis, W. R.; Colbeck, J. C.; Krebber, A.; Fleitz, F. J.; Brands, J.; Devine, P. N.; Huisman, G. J.; Hughes, G. J. *Science* **2010**, *329*, 305−309.
(8) Meadows, R. E.; Mulholland, K. R.; Schurmann, M.; Golden, M.; Kierkels, H.; May, O.; Meulenbroeks, E.; Mink, D.; Squire, C.; Straatman, H.; Wells, A. S. *Org. Process Res. Dev.* **2013**.
(9) Shin, S.-J.; Kim, B.-G. *Biotechnol. Bioeng.* **1997**, *55* (2), 348−358.