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Process Development and Scale Up of a Glycine Antagonist

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Abstract:

A synthetic route amenable to large-scale synthesis of the glycine antagonist (2*R*,4*E*)-7-chloro-4-(2-oxo-1-phenyl-pyyrrolidin-3-ylidene)-1,2,3,4-tetrahydroquinoline-2-carboxylic acid, (2*R*,3*R*,4*R*,5*S*)-6-(methylamino)hexane-1,2,3,4,5-penta-ol 12 is presented. The route consists of four stages of chemistry. Stage 1 starts from 5-chloro-2-iodoaniline hydrochloride and is a three-step telescoped stage consisting of an imine formation with ethyl glyoxalate, Mannich reaction using vinyloxytrimethylsilane, and subsequent Wittig reaction with (2-oxo-1-phenyl-3-pyrrolidinyl)triphenylphosphonium bromide. The stage 1 product (4*E*)-2[(5-chloro-2-iodophenyl)amino]-4-(2-oxo-1-phenyl-pyrrolidin-3-ylidene)butanoic acid ethyl ester 17 is subjected to an enzyme-catalysed kinetic resolution to prepare the single (2*R*)-enantiomer 19 as the ethyl ester. Stage 3 is the intramolecular Heck reaction to yield (2*R*,4*E*)-7-chloro-4-(2-oxo-1-phenyl-pyrrolidin-3-ylidene)-1,2,3,4-tetrahydroquinoline-2-carboxylic acid ethyl ester 31. The final stage is ester saponification and meglumine salt formation to afford the drug candidate molecule 12. In total, more than 300 kg of target 12 was produced with a purity >99.9%. Aspects of route selection as well as elements of process understanding and control are discussed.

Introduction

The chiral tetrahydroquinoline carboxylic acid **12** is an orally bioavailable glycine antagonist and has been identified as a potential drug candidate molecule for the treatment of nicotine craving.[1] Gram quantities of the compound were initially prepared by the discovery scientists, whereupon the scientists within Chemical Development were charged with preparing kilogram quantities of the compound to support drug safety assessment, pharmaceutical development, and clinical trials activities.

In this contribution we describe the following: (1) Our assessment of the discovery synthesis. (2) Some route evaluation work and our rationale around route selection. (3) The route of synthesis used to deliver early development supplies. (4) The route of synthesis used to deliver >300 kg of the drug candidate molecule along with aspects of process understanding and process control.

Results

The discovery route to compound **12** is shown (Scheme 1).[2] The route starts with *tert*-butyl lactate **1** which is acylated with acryloyl chloride to give **2**. Oxidative cleavage of the alkene gave the glyoxalate ester derivative **3**. The imine **5** was formed by condensation with 5-chloro-2-iodoaniline **4.**[3,4] A highly diasteroselective Lewis acid-promoted addition of allyltributyltin gave compound **6** as single diastereoisomer. A second oxidative alkene cleavage gave aldehyde **8**. An alternative sequence was to treat the imine **5** with vinyloxytrimethylsilane **7** in the presence of a catalytic quantity of Lewis acid to give directly the aldehyde **8**, but this time as an 85:15 mixture of diastereoisomers at the newly created chiral centre. Aldehyde **8** underwent a highly *E*-selective Wittig reaction upon reaction with (2-oxo-1-phenyl-3-pyrrolidinyl)triphenylphosphonium bromide **9**[5,6] to give alkene **10**. Intramolecular Heck cyclisation of alkene **10** gave the tetrahydroquinoline **11** with the exocyclic *E* double bond as the major product. The final step of the synthesis was hydrolysis of the chiral auxiliary to give the target carboxylic acid **12**. The overall sequence from *tert*-butyl lactate **1** to tetrahydroquinoline **12** using vinyloxytrimethylsilane **7** as the nucleophile was seven chemical steps and an overall yield of 26%. Initially the acid was isolated as the sodium salt. However, this salt did not possess the required pharmaceutical development attributes as it was hygroscopic and lacked sufficient photostability. During the development process the counterion was switched to the *N*-methyl-D-glucamine salt (also known as meglumine), which overcame these shortcomings.

Although the discovery routes had been used by the discovery scientists to prepare tens of grams of tetrahydroquinoline **12**, the initial drug substance requirements for chemical development scientists was several kilograms, so an assessment of the suitability of the route to provide these inflated

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(1) (a) Di Fabio, R. WO 99/64411. (b) Orlandi, A. WO 01/42238. (c) Chiamulera, C.; Reggiani, A.; Trist, D. G.; Teneggi, V. WO 2005/053693.

(2) (a) Di Fabio, R.; Alvaro, G.; Bertani, B.; Donati, D.; Giacobbe, S.; Marchioro, C.; Palma, C.; Lynn, S. M. *J. Org. Chem.* **2002**, *67*, 7319–7328. (b) Di Fabio, R.; Alvaro, G.; Bertani, B.; Donati, D.; Pizzi, D. M.; Gentile, G.; Pentassuglia, G.; Giacobbe, S.; Spada, S.; Ratti, E.; Corsi, M.; Quartaroli, M.; Barnaby, R. J.; Vitulli, G. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1176–1180.

(3) (a) Di Fabio, R.; Alvaro, G.; Bertani, B.; Giacobbe, S. *Can. J. Chem.* **2000**, *78*, 809–815. (b) Van de Lande, L. M. F. *Recl. Trav. Chim. Pay Bas.* **1932**, *51*, 98–113.

(4) Large scale quantities of 5-chloro-2-iodoaniline hydrochloride were obtained from third party suppliers and supplied as the hydrochloride salt.

(5) (a) Dolan, S. C.; Perboni, A.; Maragni, P. WO 98/39341. (b) Ikuta, H.; Shirota, H.; Kobayashi, S.; Yamagishi, Y.; Yamada, K.; Yamatsu, I.; Katayama, K. *J. Med. Chem.* **1987**, *30*, 1995–1998.

(6) Large scale quantities of (2-oxo-1-phenyl-3-pyrrolidinyl)triphenylphosphonium bromide were obtained from third party suppliers.

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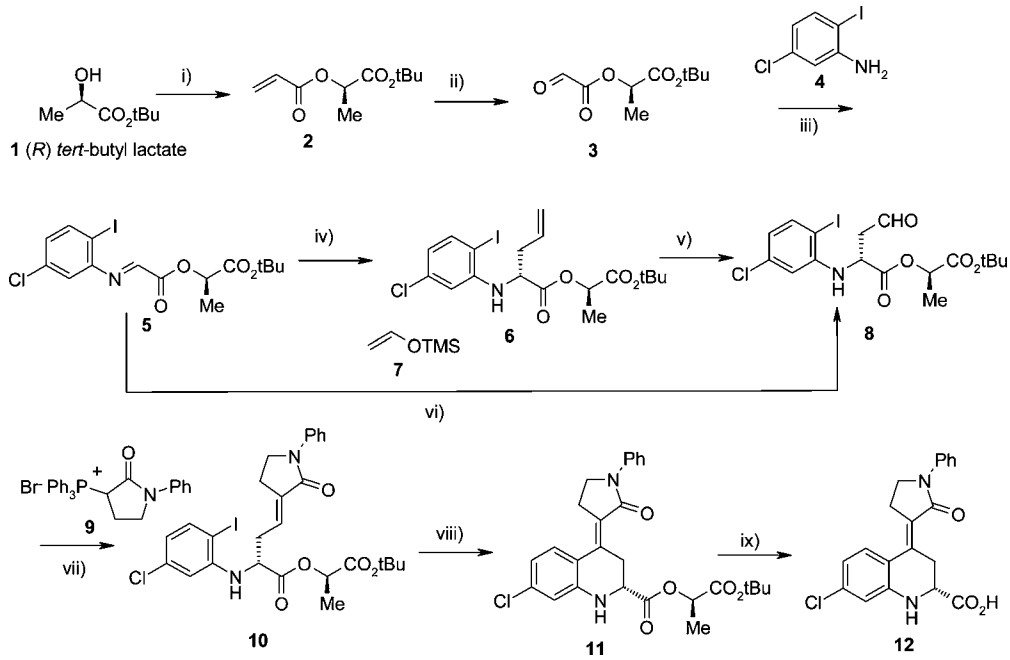
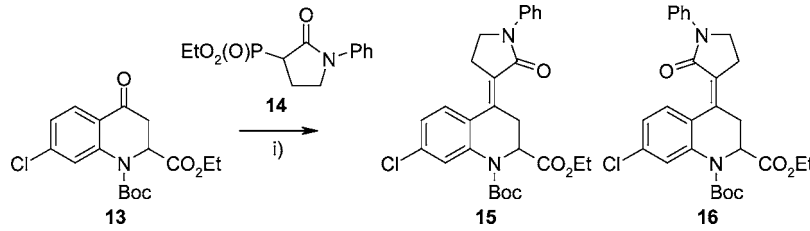
Scheme 1[a]



[a] Reagents and conditions: i) Acryloyl chloride, NEt_3, DMAP, CH_2Cl_2, 88%. ii) OsO_4, $NaIO_4$, THF, H_2O, 100%. iii) **4**, $MgSO_4$, Tol, Δ, 100%. iv) allyltributyltin, $SnCl_4$, CH_2Cl_2, −78 °C, 77%. v) O_3, PPh_3, CH_2Cl_2, −78 °C, 55%. vi) **7**, $Yb(OTf)_3$, CH_2Cl_2 −20 °C, 55%. vii) **9**, DBU, acetonitrile, −20 °C, 70%. viii) $Pd(PPh_3)_4$, NEt_3, Tol, Δ, 85%. ix) LiOH, THF, H_2O then HCl, 90%.

Scheme 2[a]



[a] Reagents and conditions: i) **14**, LiCl, DBU, acetonitrile, 50%, **15**:**16** = 5:1.

amounts was undertaken.[7] We had serious concerns about the use of the osmium and tin reagents, as they would present significant problems with respect to both worker and patient safety as well as having a serious negative environmental impact. The overall cost of goods for this route was high; one of the main contributors was the chiral auxiliary (*R*)-*tert*-butyl lactate **1**. This reagent was introduced at the beginning of the synthesis and could not be effectively recycled as it was not cleaved until the end of the synthesis. There were few solid crystalline intermediates in this route. Isolation of solid intermediates by crystallization helps to define yields but more importantly expels impurities, which is important from a quality by design perspective. From a throughput perspective the route is relatively long, seven steps, and many of the intermediates required purification by chromatography, which is both a time- and solvent-consuming unit operation.

When considering the synthesis of tetrahydroquinoline **12** there are two key synthetic features, namely the C-2 chiral centre which has the (*R*) configuration and the tetra-substituted alkene that has the (*E*) configuration.

As an alternative to the Heck reaction to form the tetrasubstituted alkene, we briefly explored some olefination strategies such as the use of Wittig reagents, e.g. **9** or the corresponding phosphonate esters, e.g. **14** (Scheme 2).[8] The best result obtained for the olefination of ketone **13** was a 50% yield of a 5:1 mixture of (*E*)-:(*Z*)-alkene isomers **15** and **16**, respectively. This was achieved using phosphonate ester **14** under the conditions described by Masamune and Roush.[9] As these preliminary results were not competitive with the use of the Heck reaction for this key transformation, we did not pursue it further and decided to focus upon routes that would incorporate the Heck reaction.

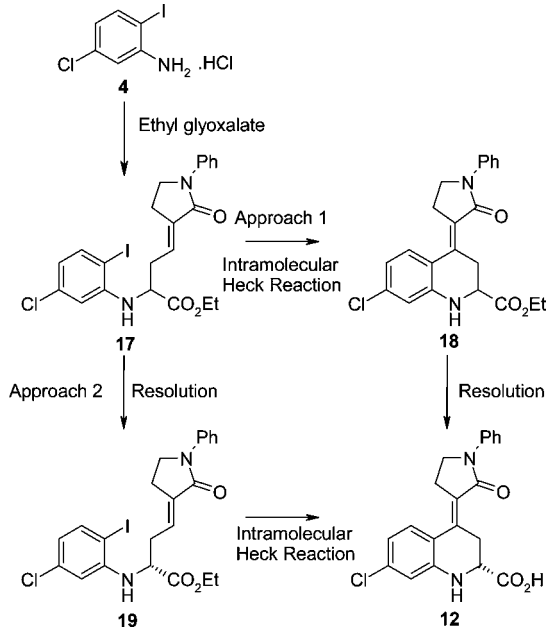
The discovery route to tetrahydroquinoline **12** relied upon a chiral auxiliary to control the addition of a nucleophile to an imine. In the case of the addition of allyltributyl stannane the levels of diastereoselectivity were excellent; the price to be paid, however, was the use of toxic tin reagents at very low

(7) Butters, M.; Catterick, D.; Craig, A.; Curzons, A.; Dale, D.; Gillmore, A.; Green, S. P.; Marziano, I.; Sherlock, J.-P.; White, W. *Chem. Rev.* **2006**, *106*, 3002–3027.

(8) Bacchi, S.; Almi, M.; Guelfi, S.; Perboni, A. 'Synthesis of 4-substituted-2-carboxy tetrahydroquinolines *via* intermolecular olefination reaction.' Poster presented at IASOC 2002 congress. September 2002, Ischia, Italy.

(9) Blanchette, M. A.; Choy, W.; Davis, J. T.; Essenfield, A. P.; Masamune, S.; Roush, W. R.; Sakai, T. *Tet. Lett.* **1984**, *25*, 2183–2186.

Scheme 3



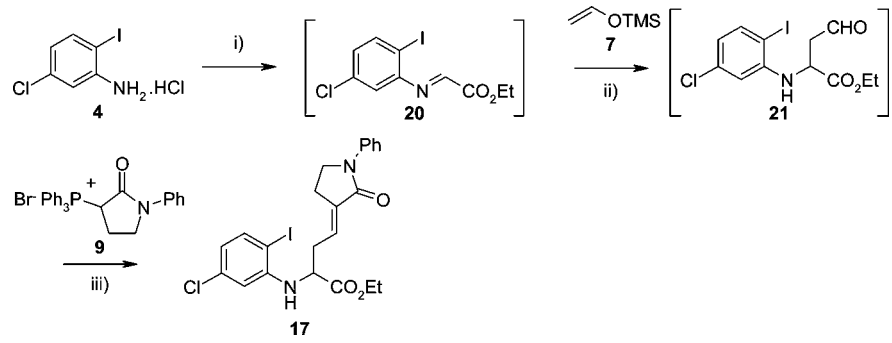
temperatures. The alternative was the addition of the vinyloxytrimethylsilane **7**, but this was a less selective reaction. In either case the isolated yield from imine **5** to aldehyde **8** was on the order of 50%. We reasoned we might be able to achieve similar yields based upon a resolution strategy and thereby use commercially available ethyl glyoxalate instead of oxalate **3**. This would reduce the number of stages and remove a potentially tricky oxidation reaction from the sequence. In addition we would look to recycle the unwanted isomer from the resolution as it was felt that the chiral centre at C-2 bearing an ester or acid function could be labile. Having made the decision about the overall route strategy (Scheme 3), there were still issues and options to be resolved as well as the short- and long-term drug substance supplies to deliver. Our first goal was a route to the racemic alkene **17**, the first intermediate on our proposed route.

The 5-chloro-2-iodoaniline **4** was obtained from the suppliers as the hydrochloride salt, and this was liberated as the free base by washing a toluene solution with aqueous potassium carbonate. To form imine **20**, aniline **4** and ethyl glyoxalate were heated to reflux under Dean and Stark conditions in the presence of magnesium sulphate (Scheme 4). On a small laboratory scale, an equilibrium mixture was established within 1 h, this mix contained 93% of the desired imine **20** along with small amounts of the following byproduct *N*,*O*-hemiaminal **22** (1%), aminal **23** (3%) and *N*,*O*-ethyl acetal **24** (2%), (Figure 1). It was assumed that the ethyl glyoxalate solution was the source of the ethanol incorporated into *N*,*O*-ethyl acetal **24**. When the reactions were scaled to a much larger scale, the final position of the equilibrium shifted, on a 112-kg scale the equilibrium position of **20** (78%): **22** (4%): **23** (6%): **24** (11%): **4** (1%) was established after 5 h. Although this equilibrium position was very different to that obtained on a small scale, it was not believed that this would have a detrimental affect on the overall efficiency of the process as it could be thought that compounds **22**, **23**, and **24** are all precursors to imine **20** under the subsequent Lewis acid conditions. After scaling the reactions to a larger scale, a revised imine-forming process was developed whereby aniline **4** and ethyl glyoxalate were heated to reflux under Dean and Stark conditions for 1 h. This established an equilibrium mix of **20** (64%): **22** (8%): **23** (24%): **24** (2%): **4** (2%), whereupon a much lower charge of magnesium sulphate (10 wt %) was added to promote the conversion to imine **20** and a final equilibrium mix of **20** (92%): **23** (3.5%): **24** (4%) was produced. The scaling up of heterogeneous reactions is often fraught with difficulties, and in the case of this imine-forming reaction it was believed that, as the scale increased, the magnesium sulphate became aggregated and adhered to the walls of the reactor, and thus was unable to participate in the process. In the revised process, aggregation of the magnesium sulphate was prevented by first removing the water, using a Dean and Stark trap, as it was produced. Many other Lewis acids were screened for their ability to promote imine formation. Of these, magnesium bromide and zinc iodide were particularly effective; however, these particular Lewis acids were not compatible with telescoping into the subsequent Mannich reaction as aldehyde **21** proved to be somewhat unstable in their presence.

The second step in the telescoped sequence was the Lewis acid-promoted Mannich addition of vinyloxytrimethylsilane **7** to imine **20** to afford aldehyde **21**. This reaction was not without difficulties as the reaction scale was increased. Initially ytterbium triflate was used to promote the transformation; however, it was found that the isolated yield was very sensitive to the reaction time. If the reaction time was much longer than 30 min, then a very low yield was obtained. This situation was felt to be unsatisfactory for scale up. The reason for this was the formation of the reactive hydroxy-tetrahydroisoquinoline **25** *via* a Friedel−Crafts reaction (Figure 2). A brief survey of Lewis acids identified trimethylsilyl triflate as a potential alternative, as the isolated yield seemed consistent regardless of reaction time. However, when this procedure was repeated on a large scale, some variability was observed. Ten batches were run on a 100 kg plus scale, and the yield (over the three steps shown in Scheme 3) varied from 47% to 62% with an average of 56%. A more in-depth investigation using process analytical techniques (PAT) was undertaken. Monitoring the reaction with either an *in situ* ReactIR probe or in a tube in an NMR machine indicated that no reaction occurred when the trimethylsilyl triflate was added to a premixed solution of imine **20** and vinyl silane **7**. Aldehyde **21** was only produced when the water, initially assumed only to quench the reaction, was added. This issue had been masked as the samples for in-process control had been quenched prior to analysis. These observations came as surprise but at least indicated the source of the variability in the process, as the *reaction* was not actually occurring until the *quench* was added. The requirement for a proton source to promote a Mannich reaction has precedent in work by Snapper and Hoveyda[10] which indicated that 1.0 equiv of isopropanol was required to be present. The possibility that triflic acid was the active promoter for the reaction was discounted as PAT monitoring of reactions containing triflic acid again showed no reaction had occurred until the water quench had been added.

(10) Josephsohn, N. S.; Snapper, M. L.; Hoveyda, A. H. *J. Am. Chem. Soc.* **2004**, *126*, 3734–3735.

Scheme 4[a]



[a] Reagents and conditions: i) Tol, aq K_2CO_3 then $MgSO_4$, EtO_2CCHO, Δ. ii) **7**, TMSOTf, −15 °C. iii) **9**, DBU, acetonitrile, 56% (over 3 steps).

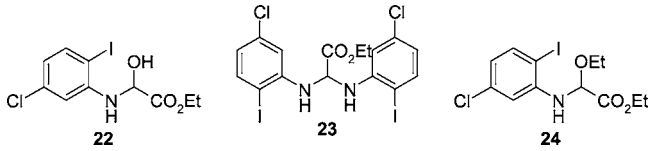


Figure 1

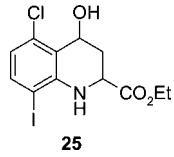


Figure 2

It would therefore appear that the reaction is actually promoted by triflic acid and a proton source, i.e., water, in the case described here. To gain greater control over the process it would be necessary to add the proton source, e.g., water, isopropanol, or aqueous acetic acid, in a controlled fashion.[11]

Although our stated aim was a route to racemic alkene **17** *via* aldehyde **21**, reports of asymmetric Mannich reactions have been published in the literature.[10,12] Our attempts to apply the reported catalysts/conditions to our system resulted in low conversions and low enantiomer excesses being obtained.[13]

The Wittig reaction between aldehyde **21** and the phosphorane formed from phosphonium salt **9** and 1,8-diazabicycl-[5.4.0]undec-7-ene proved to be straightforward. The product **17** was formed in solution as a 95:5 ratio of (*E*):(*Z*) double bond isomers. The product was then isolated from solution by way of a seeded crystallisation process from isopropanol. The product **17** was isolated as a white solid, with a purity as determined by HPLC of >99.3% PAR (peak area ratio), with the levels of the unwanted (*Z*)-isomer <0.3% PAR.

As previously mentioned, this three-step telescoped stage of chemistry was run repeatedly on a >100 kg scale, and the average yield was 56%. In total, approximately 1000 kg of alkene **17** was produced by this process.

With a concise synthesis of the carbon framework of the molecule in place, albeit in racemic form, the end-game of the synthesis had to be put in place. Here there were two choices (Scheme 3): Approach 1, perform the intramolecular Heck reaction then undergo some sort of resolution process; Approach 2, perform the resolution and then form the tetrahydroquinoline by the intramolecular Heck reaction. Our initial scale up campaign to afford approximately 10 kg of drug substance used Approach 1. This 10 kg of drug substance was used to fund key repeat dose safety assessment studies and would define the quality standard that subsequent material would have to meet. Approach 2 was used to prepare >300 kg of drug candidate for clinical studies.

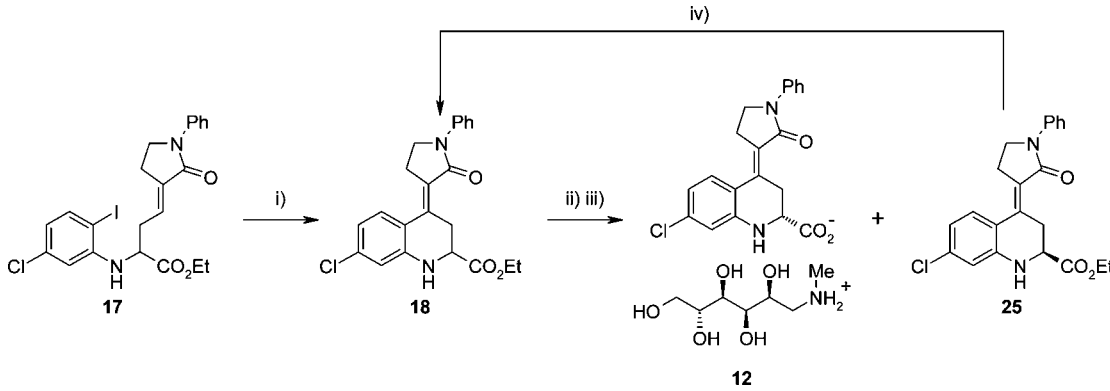
Approach 1, the Heck reaction was performed on alkene **17** using 2 mol % of $PdCl_2$ and 5 mol % triphenylphosphine, these conditions were preferable to the use of $Pd(PPh_3)_4$ as both the charge of palladium and the reaction time were both reduced (Scheme 5). The tetrahydroquinoline **18** was isolated by crystallisation in 72% yield. The isolated product did on occasion contain *ca*. 2−3% of the trans-endo isomeric byproduct **26** (Figure 3) and high levels of residual palladium, *ca*. 2000 ppm. The presence of either of these impurities did not affect the quality of the drug substance produced as they were effectively cleared in the subsequent chemistry. A resolution of the two enantiomers of **18** was achieved by way of an enzyme-catalysed hydrolysis of the ester functionality. A full screen of hydrolytic enzymes was undertaken, and from it a hit was achieved using a crude preparation from *Aspergillus niger* lipase. Further investigation showed that it was ferulic acid esterase that was the active component in the preparation and so aqueous preparations of ferulic acid esterase were obtained and used in the development of the process. The ferulic acid esterase hydrolysis of racemic ester **18** was highly selective for the hydrolysis of the (*R*)-enantiomer, with a selectivity factor $E > 100$. At the end of the reaction the desired (*R*)-enantiomer **12** could be isolated from the mixture as the meglumine salt in 37% yield, and the unreacted (*S*)-enantiomer of ester **25** could also be recovered in 55% yield. The intermediate grade drug substance was recrystallised from acetone and water to expel to low levels the oxidized quinoline impurities **27** and **28** (Figure

(11) Due to the decreased requirement for the drug candidate molecule we were unable to complete the work to gain full understanding and control of this process.

(12) (a) Kobayashi, S.; Hamada, T.; Manabe, K. *J. Am. Chem. Soc.* **2002**, *124*, 5640–5641. (b) Kobayashi, S.; Matsubara, R.; Nakamura, Y.; Kitagawa, H.; Sugiura, M. *J. Am. Chem. Soc.* **2003**, *125*, 2507–2515. (c) Cordova, A.; Barbas, C. F. *Tet. Lett.* **2003**, *44*, 1923–1926. (d) Nakamura, Y.; Matsubara, R.; Kiyohara, H.; Kobayashi, S. *Org. Lett.* **2003**, *5*, 2481–2484.

(13) Subsequent to our investigations there have been a considerable number more publications on the asymmetric Mannich reaction including examples using acetaldehyde. (a) Yang, J. W.; Chandler, C.; Stadler, M.; Kampen, D.; List, B. *Nature* **2008**, *452*, 453–454. (b) Hayashi, Y.; Okano, T.; Itoh, T.; Urushima, T.; Ishikawa, H.; Uchimaru, T. *Angew. Chem., Int. Ed.* **2008**, *47*, 9053–9058. (c) Kano, T.; Yamaguchi, Y.; Maruoka, K. *Angew. Chem., Int. Ed.* **2009**, *48*, 1838–1840.

Scheme 5[a]



[a] Reagents and conditions: i) $PdCl_2$, PPh_3, NEt_3, Tol, Δ, 72%. ii) Ferulic acid esterase, 0.1 M sodium citrate buffer, DMSO, 38 °C then aq meglumine, acetone, **12** = 37%, **25** = 55%. iii) Acetone, H_2O, 90%. iv) DBU, acetone, Δ, 88%.

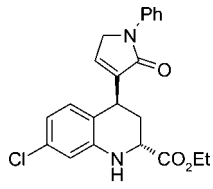


Figure 3

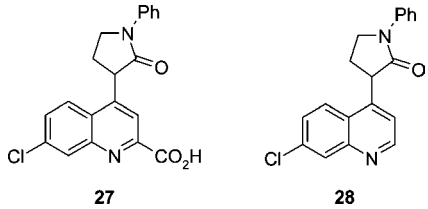


Figure 4

4). By means of the route shown in Scheme 5 14 kg of the target drug substance **12** (as meglumine salt) was prepared. The overall quality of the batches produced was excellent, area of main peak by HPLC >99.5%, all individual impurities by HPLC <0.15%, chiral purity by HPLC >99.5%, and residual palladium <10 ppm. Additionally it was shown that the unreacted (*S*)-enantiomer **26** could be recycled to the racemic compound **18** upon heating with DBU in acetone in 88% yield.

Although 14 kg of drug substance had been produced by Approach 1 as outlined in Scheme 4, it was felt there were several significant shortcomings in the process that would mean it would have been difficult to produce hundreds of kilograms *via* this route. (1) As a general rule if a classical resolution strategy is going to be employed, then it should be performed as early as possible in the synthetic sequence.[14] (2) The large workup volumes encountered in the enzymatic resolution stage severely restricted throughput. (3) Solutions of ferulic acid esterase were not freely available. (4) Although the unwanted enantiomer **25** could be recycled to give the racemic ester **18**, a buildup of impurities during this process was observed. These impurities included the trans-endo isomer **26** (which in independent experiments was shown not to be a substrate for ferulic acid esterase) as well as levels of the quinolines **27** and **28**. Producing drug substance of high quality from the recycled ester **18** was not always possible. (5) There was considerable concern over the robustness of the ferulic acid esterase process. Although the reaction was highly selective for the hydrolysis of the (*R*)-enantiomer, it was never possible to get the reaction to proceed past 40% conversion. Many different factors that could possibly affect the reaction were investigated, including temperature, enzyme loading, concentration, solvent composition, alternative buffers, and reaction pH, but none seemed to increase the level of conversion. It was the physical nature of the reaction that led to some clues as to why complete conversion was not observed. To get the reaction to proceed it was necessary to dissolve the substrate **18** in warm dimethyl sulphoxide and then to add this to a solution of the enzyme in citrate buffer. Immediately upon mixing, a precipitate formed. Further investigation by microscopy suggested that this was an amorphous coprecipitate of substrate and enzyme, and if the precipitate was filtered off and placed in a warm oven, then the reaction still proceeded. If the enzyme was challenged with crystalline substrate, then no reaction occurred. Amorphous **18** was prepared but was unstable with respect to undergoing a solid-state transition and crystallising. Subjecting amorphous **18** to the dimethyl sulphoxide/citrate buffer conditions again promoted rapid crystallisation. Our rationale for the incomplete conversion is that an amorphous coprecipitate of the enzyme and substrate is initially formed. The amorphous ester is a substrate for the enzyme, but at the same time partial crystallisation is occurring which means the reaction cannot go to completion. Since what was causing the amorphous coprecipitation was not understood or controllable, it was felt that the reaction would not be robust and not the basis of a longer-term manufacturing strategy capable of producing hundreds or thousands of kilograms.

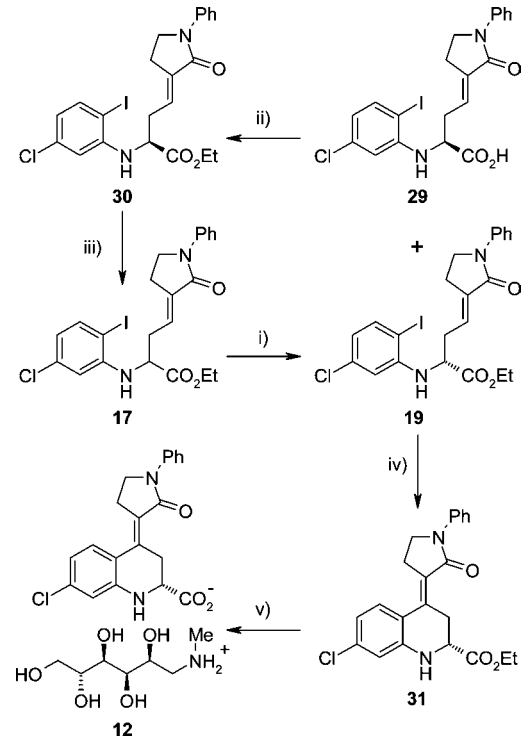
The alternative synthetic approach [Approach 2 (Scheme 3)] to perform a resolution and then carry out the intramolecular Heck reaction was investigated. Again, a resolution of the two enantiomers of **17** was achieved by way of an enzyme-catalysed hydrolysis of the ester functionality. A full screen of hydrolytic enzymes was undertaken, and from it several hits were identified. Two enzymes were identified that showed modest selectivity for the hydrolysis of the (*R*)-enantiomer of **17**. These were porcine pancreatic lipase (PPL) and trypsin derived from bovine pancreas. Additionally, two enzymes were identified that showed very high selectivity ($E > 500$) for the hydrolysis of

(14) Zang, T. *Chem. Rev* **2006**, *106*, 2583–2595.

the (*S*)-enantiomer of **17**. These were lipases from *Mucor miehei* and *Candida cylindrecea*. It was decided to try and pursue the use of *M. miehei* using the polymer-supported reagent lipozyme to develop a process. This decision was based upon the following points: (1) Both the PPL and the trypsin are derived from animal sources. To reduce the risk of the drug substance being contaminated with transmissible spongiform encaphalopathies (TSE) and therefore comply with the leading regulatory authorities it was decided not to use these reagents. (2) The enzymes PPL and trypsin only showed modest selectivity of the hydrolysis of the (*R*)-enantiomer, so a further up-grade of chiral purity would be required. (3) The enzymes *M. miehei* and *C. cylindrecea* hydrolysed the unwanted (*S*)-enantiomer, so this would mean introducing a further synthetic step to cleave the ester at the end of the synthesis. This would pose a risk that the synthetic sequence would be longer than the one shown in Scheme 5. However, it was felt that the final-stage ester hydrolysis should be a clean transformation, and therefore it should be possible to incorporate the meglumine salt formation into a final stage of chemistry and produce good-quality drug substance if the quality of the product arising from the intramolecular Heck reaction was controlled. This would eliminate the need for a final discrete recrystallisation step to upgrade product quality and therefore save a stage of chemistry.

In developing a resolution procedure for racemic ester **17** using lipozyme we looked to build upon our experience of lipase-catalysed kinetic resolutions using supported enzymes[15] (Scheme 6). Using 88% w/w *tert*-butanol[16] as solvent, the kinetic resolution could be driven to completion in *ca*. 18 h if 30% by weight of lipozyme was used. The progress of the reaction was determined by measuring the chiral purity of the reacting ester; once the ratio of enantiomers (*R*):(*S*) was >98.0:<2.0, then the reaction was deemed complete. The chiral purity of acid **29** produced during the reaction was always >99.5%. A charge of 30% by weight of lipozyme would have been uneconomical, so efforts were made to reduce the charge. As had been previously shown[15] control of pH was an effective tool, with the optimum pH for this transformation being within the range pH 6.1−6.3. As the pH of the reaction mixture decreased during the reaction, a dilute ammonia solution was added to maintain a constant pH. By controlling the pH in this way we could achieve a reaction time of <24 h using a 7% by weight charge of lipozyme. Our attempts to try and recycle the enzyme resin proved fruitless, as we showed in control experiments that the enzyme was being denatured during the process by the ethanol that was liberated. Further experimentation showed that increasing the level of *tert*-butanol present in the solvent facilitated a further reduction in lipozyme charge, without extending the reaction time. With a *tert*-butanol level of 95% w/w, the charge of lipozyme could be reduced to 3% by weight. During the scale up to 139-kg scale, we opted for the convenience of using 88% w/w *tert*-butanol.

Scheme 6[a]



[a] Reagents and conditions: i) Lipozyme, *t*-BuOH, NH_3, pH 6.2, 40 °C, 45%. ii) AcCl, EtOH, 50 °C, 44% over 2 steps. iii) NaOEt, EtOH, 0 °C, 89%. iv) $PdCl_2$, PPh_3, NEt_3, Tol, Δ, 74%. v) NaOH, aq THF, 0 °C then aq meglumine, acetone, 92%.

Once the reaction was complete, the enzyme resin was removed by filtration. Controlled addition of 0.5 equiv of aqueous sodium bicarbonate solution caused the (*R*)-ester **19** to crystallise from solution as a white solid and the (*S*)-enantiomer of acid **29** to remain in solution as the sodium salt. The (*R*)-ester **19** was consistently produced in a 45% yield, and in total, 600 kg was prepared. During the crystallisation process the chiral purity of ester **19** increased to 99.7%, and the purity as determined by HPLC was >99.5% PAR.

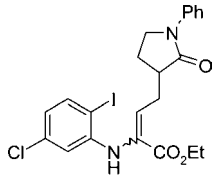
To increase the overall throughput of the sequence and to improve the economics it would be necessary to recycle the sodium salt acid **29** back to racemic ester **17**. The filtrate from the enzyme-catalysed kinetic resolution was concentrated by distillation to remove the *tert*-butanol, the sodium salt was neutralised, and the parent acid was then extracted into ethyl acetate.[17] The solvent was exchanged for ethanol, and a solution of hydrogen chloride in ethanol was added to promote the esterification to the (*S*)-ester **30**, a reaction which took *ca.* 20 h to complete at 50 °C. Ester **30** could be isolated as a white crystalline solid by seeding and then adding water. The (*S*)-ester **30** was produced in a 44% yield over two steps from racemic ester **17**, and in total, 420 kg was prepared.

Racemisation of (*S*)-ester **30** into racemic ester **17** could be achieved using sodium ethoxide in ethanol. Under the basic reaction conditions the product was prone to isomerisation to

(15) Atkins, R. J.; Banks, A.; Bellingham, R. K.; Breen, G. F.; Carey, J. S.; Etridge, S. K.; Hayes, J. F.; Hussain, N.; Morgan, D. O.; Oxley, P.; Passey, S. C.; Walsgrove, T. C.; Wells, A. S. *Org. Process Res. Dev.* **2003**, *7*, 663–675.

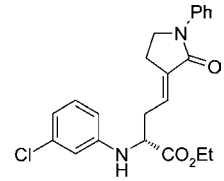
(16) 88% w/w *tert*-butanol contains by weight 88% of *tert*-butanol and 12% of water. This grade of solvent represents the azeotropic boiling mixture at atmospheric pressure, is commercially available in bulk and remains a liquid down to−10 °C.

(17) Attempts to isolate the parent acid **29** as crystalline solid were unsuccessful. Acidification of solutions of the sodium salt lead to amorphous material being produced, although this material filtered well the material lost much of its structure during drying. A crystalline potassium salt of **29** was isolated from ethyl acetate.



32

Figure 5



33

Figure 6

form compound **32** (Figure 5). This side reaction could be minimised to <2% by reducing the reaction temperature to 0 °C and reducing the charge of sodium ethoxide to 0.25 equiv. Again the reaction progress was determined by using chiral HPLC, the end point of which was determined as the ratio of (*S*)-ester **30**:(*R*)-ester **19** < 51.0:>49.0 and was achieved within 24 h. Ester **17** could be isolated as a white crystalline solid by neutralisation with AcOH and then addition of water. This recycled racemic ester **17** was produced in an 89% yield, and in total, 380 kg was prepared. The purity of recycled ester **17** as determined by HPLC was equivalent to the *de novo* ester. Importantly, when the recycled ester **17** was subjected to the lipozyme-promoted kinetic resolution, the reaction proceeded in a fashion analogous to the *de novo* material, and the quality of the (*R*)-ester **19** produced was again of equivalent quality and performance.

A modified one-pot procedure for the combined esterification and racemisation reactions was demonstrated on a small, laboratory scale. This used Amberlyst A-15 resin as catalyst for the esterification. The isolated yield of 74% was comparable with the two-stage procedure, but given the sensitivity of the racemisation protocol it was decided that a greater understanding of the reaction parameters, especially around the charge of sodium ethoxide, would be needed ahead of scale up.

The efficiency with which the resolution and racemisation protocols worked vindicated our faith in such an approach. The overall yield of (*R*)-ester **19** based upon recycling the unwanted enantiomer just the once increases from 45% to 63%; if this protocol were to go into continual manufacturing, then the overall efficiency would be 74% as the unwanted enantiomer would be continually recycled.

The second step in Approach 2 was to carry out the intramolecular Heck reaction on a single enantiomer of substrate. We had some reservation as to whether the pre-existing reaction conditions utilising excess triethylamine in refluxing toluene would be amenable to substrate **19** as both the substrate **19** and product **31** had been shown to racemise at elevated temperature in the presence of base. Fortunately, no racemisation was observed under the standard conditions used for racemic substrate **17**. As well as forming the desired product **31** the reaction also gave the identified major byproducts: the trans-endo isomer **26**, the des-halo compound **33** (Figure 6), and a small number of unidentified minor byproducts that were formed at levels <0.5% PAR. As was previously noted,[2] the product distribution obtained from this reaction depends upon the reaction conditions used. A considerable number of ligands (phosphines, palladocycles, carbenes), palladium sources, stoichiometries, solvents, and bases were screened.[18] Table 1 shows just a few selected results that show the requirement for 2.5 equiv of triphenylphosphine with respect to palladium. It was observed that the product distribution remained constant throughout the course of the reaction, thus discounting an equilibration of products once the reaction was complete. The screening experiments showed that similar results could be obtained using other high-boiling aromatic hydrocarbon solvents such as xylenes or mesitylene and that bases such as tributylamine could be used in place of triethylamine. The product distribution observed is consistent with the reaction proceeding *via* the mechanism shown in Scheme 7.[2] The origin of the reaction selectivity is not apparent, but it could be concluded that the reaction conditions used help prolong the lifetime of intermediate **34** to enable rotation to occur and then reductive elimination to occur *via* H_a rather than elimination of H_b to form the trans-endo isomer. The level of palladium chloride used was 2 mol % and that of the triphenylphosphine 5 mol %; these materials are relatively cheap, and their contribution to the overall cost of goods was so low that there was no desire to try and reduce the levels. As discussed previously, the quality of the Heck product **31** would directly affect the quality of the drug candidate molecule **12**. The two key quality-critical attributes we were concerned with were the levels of residual palladium and the level of trans-endo isomer **26**. The procedure outlined in Scheme 5 was used to fund the key repeat dose safety assessment studies and therefore, in conjunction with the ICH guidelines, set the bench mark for quality for future material. That route was tolerant of levels of palladium and trans-endo isomer **26** much greater than those in Scheme 6. Many palladium scavengers have been reported in the litera-

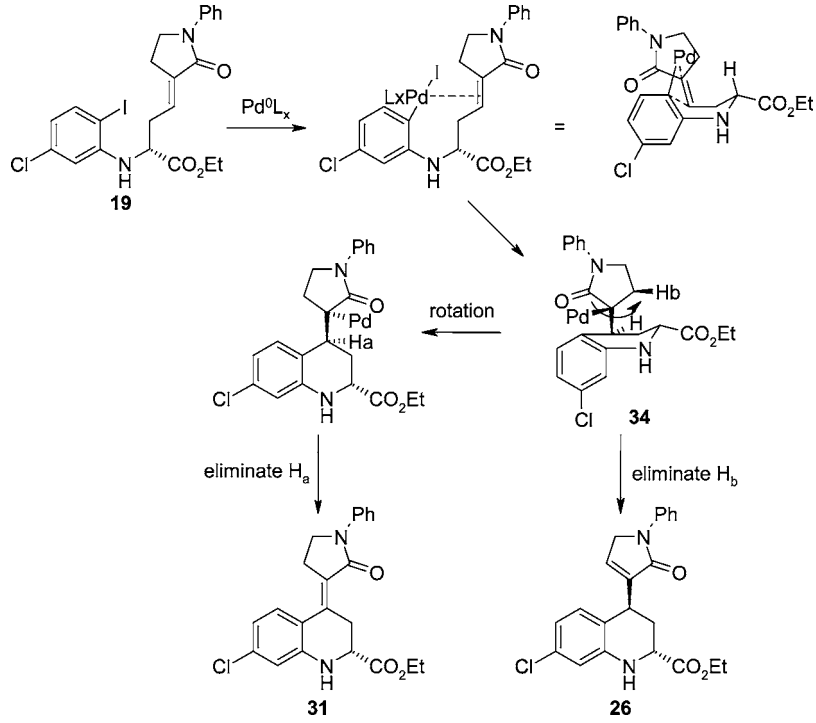
Table 1. **Effect of solvent and ligand on intramolecular Heck reaction**

PPh_3 (equiv)	solvent	yield (%)	ratio of **31**:**26**:**33**
0	toluene	60	17:78:3
0	DMF	70	10:82:8
1	toluene	97	83:11:6
2.0	toluene	>99	85:6:8
2.5	toluene	>99	88:7:5
2.5	DMF	97	31:61:8
4	toluene	>99	88:7:5

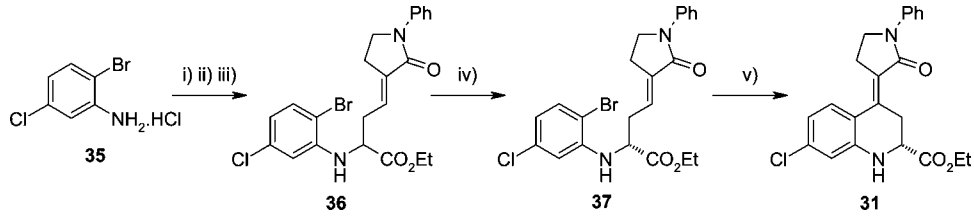
[a] Peak area ratios determined by HPLC at 245 nm.

(18) (a) Amatore, C.; Jutand, A. *Acc. Chem. Res.* **2000**, *33*, 314–321. (b) Beletskaya, I. P.; Cheprakov, A. V. *Chem. Rev.* **2000**, *100*, 3009–3066. (c) Whitcombe, N. J.; Hii, K. K.; Gibson, S. E. *Tet.* **2001**, *57*, 7449–7476. (d) Littke, A. F.; Fu, G. C. *Angew. Chem., Int. Ed.* **2002**, *41*, 4176–4211. (e) Prashad, M. *Top. Organomet. Chem.* **2004**, *6*, 181–203. (f) Farina, V. *Adv. Synth. Catal.* **2004**, *346*, 1553–1582.

Scheme 7



Scheme 8[a]



[a] Reagents and conditions: i) Tol, aq K_2CO_3 then $MgSO_4$, EtO_2CCHO, Δ. ii) **7**, TMSOTf, −15 °C. iii) **9**, DBU, acetonitrile, 60% (over 3 steps). iv) Lipozyme, *t*-BuOH, NH_3, pH 6.2, 40 °C, 40%. v) $PdCl_2(Ph_3)_2$, aq K_2CO_3, 1,4-dioxane, Δ.

ture;[19] it was found that the addition of 6 mol % of trimercaptotriazine[20] at the end of the reaction and then continuing to heat at reflux reduced the levels of residual palladium in ester **31** from *ca.* 2000 ppm to <15 ppm. The excess trimercaptotriazine and the palladium−trimercaptotriazine complex could be removed by filtration along with triethylammonium iodide. After an aqueous workup the ester **31** could be isolated from a toluene solution by the controlled addition of isooctane. On an 87 kg input scale, ester **31** was consistently isolated in 74% yield, and in total, 245 kg were produced. The quality of the intermediate was excellent, no des-halo **33** was detected, and the levels of the trans-endo isomer **26** were in the range 0.16−0.21% PAR, the target specification for this impurity being <0.25% PAR. No racemisation of the chiral centre was observed during the reaction, and the chiral purity of the isolated product was 100%.

There are aspects of quality by design that could be incorporated into this stage of chemistry. One such example would be the use of the commercially available preformed bis(triphenylphosphine)palladium(II) dichloride catalyst. Given the sensitivity of the reaction outcome to the ratio of palladium to ligand, it would increase the consistency of the reaction in terms of both profile and duration if only a single reagent needed to be charged and the ratio of palladium to ligand to be fixed. It should be noted that the ratios listed in Table 1 are not normalised for the relative response of each component, so any fine-tuning of the reaction profile would need quantitative analysis to maximise the amount of desired product **31** being formed. Since impurities are going to be produced, then the impurity that is easiest to expel (in this case the des-halo compound **33**) should be formed in preference to the trans-endo compound **26** which is more difficult to remove.

An analogous route using the bromo-substituted Heck precursor was also investigated briefly, Scheme 8. The Heck reaction precursor **37** was prepared from 2-bromo-5-chloroaniline **35**[3b] as a single enantiomer by applying the methods used in Schemes 4 and 6. The conditions used for ester **19** were not suitable for this substrate. Using triphenylphosphine as ligand a screen of solvents and bases showed that a product distribution of desired product **31**:trans-endo isomer **26**:des-

(19) (a) Galaffu, N.; Man, S. P.; Wilkes, R. D.; Wilson, J. R. H. *Org. Process Res. Dev.* **2007**, *11*, 406–413. (b) Pink, C. J.; Wong, H.-T.; Ferreira, F. C.; Livingston, A. G. *Org. Process Res. Dev.* **2008**, *12*, 589–595. (c) Flahive, E. J.; Ewanicki, B. L.; Sach, N. W.; O'Neill-Slawecki, S. A.; Stankovic, N. S.; Yu, S.; Guinness, S. M.; Dunn, J. *Org. Process Res. Dev.* **2008**, *12*, 637–645, references therein.

(20) Rosso, V. W.; Lust, D. A.; Bernot, P. J.; Grosso, J. A.; Modi, S. P.; Rusowicz, A.; Sedergran, T. C.; Simpson, J. H.; Srivastava, S. K.; Humora, M. J.; Anderson, N. G. *Org. Process Res. Dev.* **1997**, *1*, 311–314.

halo compound **33** of 86:9:0.5 could be achieved using potassium carbonate as base and dioxane as solvent. Again no racemisation at C-2 was observed.

The final stage in the synthetic sequence required saponification of the ethyl ester **31** to the carboxylic acid **12**, formation of the meglumine salt, and then a crystallisation that controlled not only the chemical purity but also the solid-state attributes as well. The quality attributes of ester **31** had already been controlled, that is chiral purity, related impurities by HPLC, and residual Pd. It was felt that only the chiral purity could be eroded during the final processing step. To refine and optimize the reaction conditions a statistical design of experiment (DoE) approach was used; the factors studied were reaction temperature (range 0−20 °C), amount of sodium hydroxide, volumes of tetrahydrofuran and water, and the rate at which the aqueous sodium hydroxide was added. The most significant factor affecting product quality was the reaction temperature, with the low temperature being optimum. The other factors were then modified to ensure that the reaction was complete within 4 h and that a homogeneous reaction solution was obtained at the end point. Under the optimised reaction conditions only 0.3% racemisation was observed. No upgrade of chiral purity was observed during the crystallisation of form 2. Once the reaction was complete, the excess sodium hydroxide was neutralized with hydrochloric acid, and the parent free acid **12** was extracted and solvent exchanged into acetone. The meglumine salt was formed by addition of aqueous *N*-methyl-D-glucamine, the solution composition was adjusted to make the solution metastable with respect to the salt, and then a seed of form 2 was added to induce crystallisation which was followed by addition of further acetone antisolvent and temperature lowering to increase recovery. Two solid-state forms of salt **12** were known. Form 1 was a metastable channel hydrate, while form 2 was a highly crystalline, nonsolvated, nonhydrated form; it was form 2 that was required. On a 50 kg input scale, salt **12** was consistently isolated in 92% yield; in total, >300 kg were produced. The quality of the final drug candidate molecule was excellent, with the main peak >99.9% PAR; no related impurities were detected above 0.1% PAR, the chiral purity was 99.7%, the level of residual Pd <10 ppm, and the crystalline form was always form 2.

Following Approach 2 as outlined in Scheme 3 and as exemplified in Scheme 6, >300 kg of the chiral tetrahydroquinolone **12** was produced. This approach overcame many of the shortcomings outlined with Approach 1. The resolution was performed earlier in the synthetic sequence, and the unwanted enantiomer was recycled to produce material of quality similar to that of the *de novo* material; this improved throughput and reduced cost. By controlling the quality of the penultimate intermediate **31**, the quality of the final compound obtained met all specifications without the need for a recrystallisation, and this kept the number of stages of chemistry to a minimum.

Conclusions

In conclusion, in this paper we have described our Chemical Development effort to support a glycine antagonist to be used for treatment of nicotine craving. The development chemists delivered >300 kg of drug candidate molecule using two related routes of synthesis. The development routes used a resolution strategy, whereas the discovery route used a chiral auxiliary approach; by recycling the unwanted enantiomer the throughput and economics could be increased and potentially problematic oxidation steps removed. By performing the resolution strategy earlier in the sequence, improved throughput was achieved, and a more readily available supported enzyme could be used. Using PAT afforded greater process understanding of the Mannich reaction to form aldehyde **21**, but due to resourcing constraints a complete solution to the problem could not be obtained. The quality attributes of the target compound **12** could be achieved by control of the quality of the penultimate intermediate **31**, controlling the reaction temperature during the saponification, and by controlling the final crystallisation.

Experimental Section

(4*E*)-2[(5-Chloro-2-iodophenyl)amino]-4-(2-oxo-1-phenylpyrrolidin-3-ylidene)butanoic Acid Ethyl Ester (17). 5-Chloro-2-iodoaniline hydrochloride (112 kg, 386 mol) was suspended in toluene (927 kg) and treated with a solution of potassium carbonate (56 kg, 405 mol) in water (560 L) at 20−25 °C for 30 min. The phases were separated, and the organic phase was washed with water (560 L). Anhydrous magnesium sulphate (84.0 kg, 699 mol) was added and the solution dried by heating at reflux with a Dean and Stark trap. A 50% w/w solution of ethyl glyoxalate in toluene (119 kg, 582 mol) was added over 30 min, maintaining reflux. The reaction mixture was maintained at reflux for 5 h and water (8.8 L) collected in the Dean and Stark trap. The reaction mixture was cooled to −15 °C, and vinyloxytrimethylsilane (67 kg, 578 mol) was added over 10 min. Trimethylsilyl triflate (4.3 kg, 19.3 mol) was added over 5 min maintaining the temperature at −15 to −19 °C. After stirring for 30 min water (560 L) was added, allowing the temperature to rise to 20−25 °C. The phases were separated, and the organic phase was washed with water (560 L). The organic phase was filtered to remove residual inorganic solids and then concentrated by distillation under reduced pressure, maintaining the internal temperature below 60 °C to leave a solution of aldehyde **21** with a residual volume of 780 L.

Phosphonium salt **7** (195 kg, 388 mol) was suspended in acetonitrile (386 kg) and 1,8-diazabicyclo[5.4.0]undec-7-ene (63 kg, 414 mol) added; the mixture was stirred at 18−22 °C for 1 h and 40 min to form a solution of the phosphorane. The toluene solution of aldehyde **21** (780 L) was added to the phosphorane solution over 50 min. The mixture was stirred for 45 min, and then water (560 L) was added. After stirring for 15 min the phases were separated, and the organic phase was concentrated by distillation under reduced pressure to leave a residual volume of 370 L. Isopropanol (704 kg) was added and the temperature adjusted to 28 °C. Seed crystals of alkene **17** (125 g) were added, and the solution was aged at 25−30 °C until crystallisation was established. The slurry was cooled to 22 °C, aged for 2 h, then cooled and aged at 0 °C for 1 h. The product was isolated by filtration, and the filtercake was washed with isopropanol (354 kg) and then dried under vacuum at 45−50 °C to afford alkene **17** as a white solid (113 kg, 56% yield over three steps).

Mp = 83−85 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (m, 2 H), 7.56 (m, 1 H), 7.39 (m, 2 H), 7.16 (tt, J = 7.3, 1.1 Hz,

1 H), 6.58 (tt, $J = 7.7$, 2.8 Hz, 1 H), 6.51−6.49 (m, 2 H), 4.87 (d, $J = 8.0$ Hz, 1 H), 4.29−4.24 (m, 3 H), 3.88 (t, $J = 6.9$ Hz, 2 H), 2.85−2.73 (m, 4 H), 1.30 (t, $J = 7.1$ Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 166.5, 146.5, 139.9, 139.6, 136.2, 135.6, 128.9, 127.1, 124.7, 119.7, 119.6, 111.1, 83.05, 62.0, 55.8, 45.3, 32.1, 21.7, 14.3; IR (neat cm^{-1}): 3361, 1730, 1690, 1667. LRMS (CI + ve) *m*/*z* 525 ($M^+ + H$).

(2*R*,4*E*)-7-Chloro-4-(2-oxo-1-phenyl-pyrrolidin-3-ylidene)-1,2,3,4-tetrahydroquinoline-2-carboxylic Acid, (2*R*,3*R*,4*R*,5*S*)-6-Methylamino-hexane-1,2,3,4,5-penta-ol (12) - Scheme 5. Ester **18** (10.0 kg, 25.22 mol) was dissolved in dimethyl sulphoxide (55 L) at 50 °C and then cooled to 40 °C. The ester solution was added to a solution of ferulic acid esterase (4 L; 4% w/w; aq) in sodium citrate buffer (125 L; 0.1 M; aq) and dimethyl sulphoxide (20 L) at 35 °C, and the resulting slurry was stirred at 38 °C for 20 h. Butanone (200 L) was added, the solution was cooled to 20−25 °C, and the phases were separated. To the aqueous layer was added brine (50 L; 20% w/w; aq), and then the mixture was extracted with butanone (100 L). The butanone extracts were combined and then washed with brine (160 L; 6% w/w; aq), brine (200 L; 6% w/w; aq), and then brine (80 L; 20% w/w; aq). Further butanone (100 L) was added and the solution concentrated by distillation at atmospheric pressure to leave a residual volume = 40 L. Acetone (270 L) was added followed by a solution of *N*-methyl-D-glucamine (2.46 kg, 12.61 mol) in water (12 L) at 20 °C. The resulting slurry was aged at 20 °C for 1 h. The product was isolated by filtration, and the filtercake was washed with acetone (100 L) and then dried under vacuum at 40 °C to afford intermediate grade salt **12** as a yellow solid (5.27 kg, 37%).

The mother liquors and wash containing ester **25** were concentrated by distillation at atmospheric pressure to leave a residual volume = 100 L. Water (185 L) was added at 20 °C and the resulting slurry aged at 20 °C for 1 h. The product was isolated by filtration, and the filtercake was washed with water (50 L) and then dried under vacuum at 50 °C to afford ester **25** as a yellow solid (4.50 kg, 45%).

The intermediate grade salt **12** (7.90 kg, 14.0 mol) was suspended in water (24 L) and warmed to 55 °C to achieve complete dissolution and then filtered into a clean reactor, and the lines were rinsed through with water (8 L). The solution was cooled to and maintained at 40 °C, and acetone (95 L) was added *via* a filter. Seed crystals of product **12** (form 2, 16 g) were added. Acetone (190 L) was added *via* a filter, and then the resulting slurry was cooled to 20 °C and aged for 1 h. The slurry was cooled to 2 °C and aged for 1 h. The product was isolated by filtration, and the filtercake was washed with acetone (2 × 40 L) and then dried under vacuum at 50 °C to afford salt **12** as a yellow solid (7.11 kg, 90%).

(2*R*,*S*,4*E*)-7-Chloro-4-(2-oxo-1-phenyl-pyrrolidin-3-ylidene)-1,2,3,4-tetrahydroquinoline-2-carboxylic Acid Ethyl Ester (18) - Scheme 5. Ester **25** (200 g, 504 mmol) was suspended in acetone (1.4 L), and then 1,8-diazabicycl[5.4.0]undec-7-ene (18.8 mL, 126 mmol) was added. The mixture was heated at reflux for 4 h and then cooled to 40 °C, and acetone (1.0 L) was added. The mixture was then cooled to 20−25 °C and hydrochloric acid (1.0 L; 0.1 M; aq) added over 20 min. The resulting slurry was aged and then filtered, and the filtercake was washed with acetone/water (400 mL; 1:1 v/v). The filtercake was dried to leave ester **18** as a yellow solid (175 g, 88%).

(2*R*,4*E*)-2[(5-Chloro-2-iodophenyl)amino]-4-(2-oxo-1-phenyl-pyrrolidin-3-ylidene)butanoic Acid Ethyl Ester (19) - Scheme 6. Racemic ester **17** (139 kg, 265 mol) and lipozyme RM IM-supported enzyme (9.8 kg,) were slurried in 88% w/w *tert*-butanol (1239 kg) at 39−41 °C, and the pH was maintained at pH 6.1−6.3 by the addition of a solution of 1.5 M ammonia in 88% w/w *tert*-butanol (typically 73.5 L). Once the reaction was complete (as measured by chiral HPLC: (*R*)-ester **19**:(*S*)-ester **30** > 98.0:2.0, time 19 h), the resin was removed by filtration and washed with 88% w/w *tert*-butanol (113 kg). To the combined filtrates at 40 °C was added sodium bicarbonate (427 kg; 2.6% w/w; aq; 132 mol). The mixture was stirred at 40 °C for 1 h during which period ester **19** crystallized from solution. The slurry was cooled to 5 °C over 2 h 30 min and then was aged at 3−4 °C for 1 h. The product was isolated in a centrifuge and was washed with a mixture 88% w/w *tert*-butanol (113 kg) and water (139 L) and then dried under vacuum at 40−45 °C to afford ester **19** as a white solid (63 kg, 45% yield).

The mother liquors and cake wash, containing acid **29** as the sodium salt, were concentrated by distillation under reduced pressure to leave a residual volume = 485 L. The concentrate contained approximately 62 kg of acid **29**.

HPLC in process control method: Chiracel OD column 250 mm × 4.6 mm, 5 μm. Eluent: 80% *n*-heptane, 20% EtOH + 0.1% TFA. Isocratic for 20 min. Flow rate 1.0 mL/min. Detector at 255 nm. Injection volume 20 uL. Typical retention times: (*R*)-enantiomer of acid, 7.4 min; (*S*)-acid **29**, 8.0 min; (*S*)-ester **30**, 9.4 min; (*R*)-ester **19**, 10.0 min.

(2*S*,4*E*)-2[(5-Chloro-2-iodophenyl)amino]-4-(2-oxo-1-phenyl-pyrrolidin-3-ylidene)butanoic Acid Ethyl Ester (30) - Scheme 6. To a reactor were charged a solution of sodium salt of acid **29** (1100 kg; *ca*. 13% w/w; aq; 283 mol) and ethyl acetate (1130 kg). Hydrochloric acid (115 kg; 2 M; aq) was added, and pH of aqueous layer = 6.0−6.5. The layers were separated, and then the organic layer was concentrated by distillation at atmospheric pressure to leave a residual volume = 265 L. Ethyl acetate (890 kg) was added and then the solution concentrated by distillation at atmospheric pressure to leave a residual volume = 320 L. Ethanol (500 kg) was added to give a solution of acid **29** and the temperature adjusted to 45−50 °C. To a separate reactor was charged ethanol (110 kg), and acetyl chloride (15.4 kg, 196 mol) was added over 40 min, maintaining temperature <35 °C. The solution of hydrogen chloride in ethanol was added to the solution of acid **29** in ethanol and then continued stirring at 45−50 °C until the reaction was complete (as measured by HPLC: ester **30**:acid **29** > 95.0:5.0, time 20 h). The solution was cooled to 17−22 °C, and seed crystals of ester **30** (25 g) were added. The slurry was cooled to 8−10 °C, and then water (315 L) was added. The slurry was further cooled to 0−5 °C and aged for 1 h. The product was isolated by filtration, and the filtercake was washed with ethanol/water (265 kg; 3:1 v/v) and then dried under vacuum at 45−50 °C to afford ester **30** as a white solid (141 kg, 44% yield over two steps).

(2*R*,*S*,4*E*)-2[(5-Chloro-2-iodophenyl)amino]-4-(2-oxo-1-phenyl-pyrrolidin-3-ylidene)butanoic Acid Ethyl Ester (17) - Scheme 6. Ester **30** (140 kg, 267 mol) was suspended in ethanol (540 kg) at −2 to +2 °C. Sodium ethoxide in ethanol (21.6 kg; 21% w/w; 67 mol) was added, maintaining the temperature within the range −2 to +2 °C and then continued stirring at this temperature until the reaction was complete (as measured by chiral HPLC: (*S*)-ester **30**:(*R*)-ester **19** < 51.0: 49.0, time 24 h). Acetic acid (4.0 kg, 67 mol) was added and the slurry stirred for 30 min. Water (140 L) was added and the slurry aged at −2 to +2 °C for 30 min. The product was isolated by filtration, the filtercake was washed with ethanol/water (160 kg; 78:22 w/w) and then dried under vacuum at 45−50 °C to afford ester **17** as a white solid (125 kg, 89% yield).

(2*R*,4*E*)-7-Chloro-4-(2-oxo-1-phenyl-pyrrolidin-3-ylidene)-1,2,3,4-tetrahydroquinoline-2-carboxylic Acid Ethyl Ester (31) - Scheme 6. To a reactor were charged ester **19** (87 kg, 166 mol), triphenylphosphine (2.177 kg, 8.3 mol), palladium(II) chloride (0.589 kg, 3.32 mol) and toluene (1060 kg). Triethylamine (22.3 kg, 220 mol) was added, and the mixture was heated at reflux until the reaction was complete (as measured by HPLC: alkene **17**:ester **19** < 1.0:99.0, time 3 h 45 min). Trimercaptotriazine (1.74 kg, 9.81 mol) was added and mixture heated at reflux for a further 1 h. The solution was cooled to 45 °C, and the solids were removed by filtration. The filtercake was washed with toluene (152 kg). With the temperature maintained at 40−45 °C, the combined filtrates were washed twice with water (2 × 696 L) before being concentrated by distillation under reduced pressure to leave a residual volume = 680 L. The concentrate temperature was adjusted to 45 °C, and isooctane (602 kg) was added over 1 h 40 min whilst maintaining the temperature at 41−44 °C. The resulting slurry was cooled and aged at 18 °C for 2 h. The product was isolated by filtration, the filtercake was washed with heptane/isooctane (2 × 177 L, 50:50 v/v) and then dried under vacuum at 45−50 °C to afford ester **31** as a yellow solid (48.7 kg, 74% yield).

Mp = 160−162 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (m, 2 H), 7.39 (m, 2 H), 7.17 (tt, J = 7.3, 1.0 Hz, 1 H), 7.14−7.12 (m, 1 H), 6.65−6.63 (m, 2 H), 4.79 (s, 1 H), 4.22−4.03 (m, 4 H), 3.85 (ddd, J = 9.1, 7.8, 6.0 Hz, 1 H), 3.77 (ddd, J = 9.1, 7.9, 4.9 Hz, 1 H), 3.48−3.42 (m, 1 H), 3.17−3.12 (m, 2 H), 1.17 (t, J = 7.1 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4, 168.3, 145.7, 139.8, 137.6, 135.7, 129.1, 128.9, 125.2, 124.6, 119.8, 118.3, 116.8, 114.4, 61.6, 53.9, 45.6, 28.0, 25.2, 14.1; IR (neat cm^{-1}): 3369, 1711, 1664; LRMS (CI + ve) m/z 397 (M^+ + H).

(2*R*,4*E*)-7-Chloro-4-(2-oxo-1-phenyl-pyrrolidin-3-ylidene)-1,2,3,4-tetrahydroquinoline-2-carboxylic Acid, (2*R*,3*R*,4*R*,5*S*)-6-Methylamino-hexane-1,2,3,4,5-penta-ol (12) - Scheme 6. Ester **31** (48.7 kg, 123 mol) was dissolved in tetrahydrofuran (300 kg) and demineralised water (161 L) and was cooled to 0 °C. A solution of sodium hydroxide (78 L; 10% w/w; aq; 195 mol) was added, whilst maintaining the temperature at 1−2 °C. The mixture was stirred at −1 to +1 °C until the reaction was complete (as measured by HPLC: ester **31**:acid **12** < 1.0:99.0, time 2 h 45 min). Hydrochloric acid (78 L; 2.5 M; aq; 195 mol) was added, maintaining the temperature at 2 °C. Dichloromethane (519 kg) was added, the solution was warmed to 20−25 °C and the phases separated. The aqueous phase was extracted with dichloromethane (259 kg), then the combined organic extracts were washed with demineralised water (195 L) before being filtered into a clean reactor and concentrated by distillation under reduced pressure to leave a residual volume = 146 L. Acetone (308 kg) was added *via* a filter and the solution was concentrated by distillation under reduced pressure to leave a residual volume = 146 L. The temperature of the concentrate was adjusted to 42 °C, and a prewarmed solution of *N*-methyl-D-glucamine (23.9 kg, 123 mol) in demineralised water (122 L) was added *via* a filter. Acetone (78 kg) was added *via* a filter, the temperature was adjusted to 41 °C, and seed crystals of product **12** (form 2, 245 g) were added. Acetone (577 kg) was added *via* a filter and the slurry stirred at 40 °C for 1 h. The slurry was cooled to 4 °C and aged for 1 h. The product was isolated by filtration, and the filtercake was washed with acetone (2 × 77 kg) and then dried under vacuum at 35−40 °C to afford salt **12** as a yellow solid (63.9 kg, 92% yield).

Mp = 186 °C; Anal. Calcd for $C_{20}H_{17}ClN_2O_3 \cdot C_7H_{17}NO_5$: C, 57.50; H, 6.08; N, 7.45; Cl, 6.29. Found: C, 57.5; H, 5.8; N, 7.3; Cl, 6.1. ^{1}H NMR (400 MHz, D_2O) δ 7.58−7.44 (m, 4 H), 7.31 (t, J = 6.9 Hz, 1 H), 7.23 (d, J = 8.8 Hz, 1 H), 6.78 (d, J = 2.0 Hz, 1 H), 6.68 (dd, J = 8.4, 1.9 Hz, 1 H), 4.10 (m, 1 H), 3.92 (t, J = 5.8 Hz, 1 H), 3.89−3.72 (m, 5 H), 3.72−3.61 (m, 3 H), 3.31 (dd, J = 13.9, 4.5 Hz, 1 H), 3.23 (dd, J = 13.0, 3.6 Hz, 1 H), 3.19 (dd, J = 13.0, 9.3 Hz, 1 H), 3.08 (m, 2 H), 2.77 (s, 3 H); ^{13}C NMR (100 MHz, D_2O) δ 181.5, 172.2, 148.0, 140.4, 140.2, 136.6, 131.0, 130.6, 127.6, 126.3, 123.7, 119.9, 117.5, 115.6, 72.3, 72.1, 72.0, 69.5, 64.1, 57.4, 52.5, 48.5, 34.4, 29.6, 27.9; IR (neat cm^{-1}) 3392, 1737, 1218; HRMS calcd for $C_{20}H_{17}ClN_2O_3$ + H^+ 369.1005, found 369.0995;

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Supporting Information Available

^{1}H and ^{13}C NMR spectra for compounds **26**, **27**, and **28**. This material is available free of charge via the Internet at http://pubs.acs.org.

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