Organic Process Research & Development

Communication

pubs.acs.org/OPRD

A Design of Experiments Approach to a Robust Final Deprotection and Reactive Crystallization of IPI-926, A Novel Hedgehog Pathway Inhibitor

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Supporting Information

ABSTRACT: A design of experiments (DoE) approach was taken to optimize purity and reaction yield of the final debenzylation and hydrochloride salt formation of IPI-926. The study involved a careful dissection of the different process steps to enable an independent investigation of these steps while ensuring that process streams were representative. The results enabled a streamlined process from the final chemical transformation to the salting and isolation and led to the elimination of variability in the process as well as a robust control of impurities. The optimized process was applied to production and demonstrated on the kilogram scale.

INTRODUCTION

The hedgehog pathway inhibitor IPI-926[1] has been in clinical investigation for basal cell carcinoma, chondrosarcoma, and pancreatic cancer. In the final step of the synthesis of IPI-926 (Scheme 1) the drug substance (DS) is isolated as the hydrochloride salt of the 2-propanol (2-PrOH) solvate. The manufacture of the DS involves the debenzylation of compound **1** to form the IPI-926 free base, which is then salted after telescoping the two steps via a solvent exchange. This report outlines the approach taken for first enabling then optimizing the two-step telescoped process.

The application of multivariate methods and particularly Design of Experiments (DoE) approaches to chemical development have been successfully used for several years. More recently, the practice has been acknowledged by regulatory agencies within the concept of Quality by Design (QbD), and it is becoming the cornerstone of any robust process development strategy within the industry, from Drug Substance to Drug Product, and beyond.

Applying a DoE approach, the strategy for the optimization in the manufacture of IPI-926 was to look at each step of the process independently to probe its impact on the overall process. Namely, the goal was to optimize the debenzylation, metal removal, and salting steps one-at-a-time at first, then as a complete sequence. This approach was chosen out of consideration for the complexity of the operations (to break the stage into manageable unit operations), as well as because it was believed that factors influencing the debenzylation reaction would not necessarily influence the salting steps. In all cases where DoE was used, an optimal design[2] with response surface approach was applied, which minimizes confounding and allows to evaluate all main effects, quadratic terms, and cross terms efficiently.

DEPROTECTION OPTIMIZATION

Initial Approach. The debenzylation of compound **1** to generate IPI-926 was first successfully performed with hydrogen gas using $Pd(OH)_2$ in ethanol (EtOH). After preliminary catalyst and solvent screens, it was determined that Pd/C in either 2-methyltetrahydrofuran (2-MeTHF) or a 4:1 (v/v) mixture of 2-MeTHF and 2-PrOH was the best combination of solvent and catalyst for this reaction. All of the reactions in the initial screen were performed at room temperature and using atmospheric (1 bar) hydrogen.

A more advanced catalyst screen was then undertaken, with the specific aim of optimizing the catalyst system with the following constraints: (1) > 99% conversion required in <5 h reaction time; (2) <5% reaction byproducts (total by HPLC or ^{1}H NMR); (3) <5 bar H_2 pressure to avoid specialized equipment; and (4) minimized catalyst loading. From this screen, the best catalyst was determined to be Johnson-Matthey A405032-5 (Pd/C).[3]

Design of Experiments. Once the catalytic system was selected, a DoE[4] approach using a D-Optimal design was taken to investigate the effect of reaction (1) catalyst loading (Pd), (2) temperature, and (3) hydrogen pressure on the conversion and byproducts after a reaction time of 1 h.[5] The design was duplicated at two different concentrations (5% g/mL, or 20 vol.; 10% g/mL, or 10 vol.) to probe the effect of this variable on the reaction conversion (Table 1).

Analysis of the data from these 32 experiments determined substrate concentration, catalyst loading, and temperature to be statistically significant (model fit $p < 0.0001$; R^2 Adj 0.91) to the measured response (conversion). Higher temperature, catalyst loading, and substrate concentration all resulted in better conversion. However, catalyst loading and temperature were found to have the most profound effect on conversion (Table

Special Issue: Application of Design of Experiments to Process Development

Received: June 29, 2015

DOI: 10.1021/acs.oprd.5b00214
Org. Process Res. Dev. XXXX, XXX, XXX−XXX

Scheme 1. Synthesis of IPI-926 and Salt Formation from Compound 1

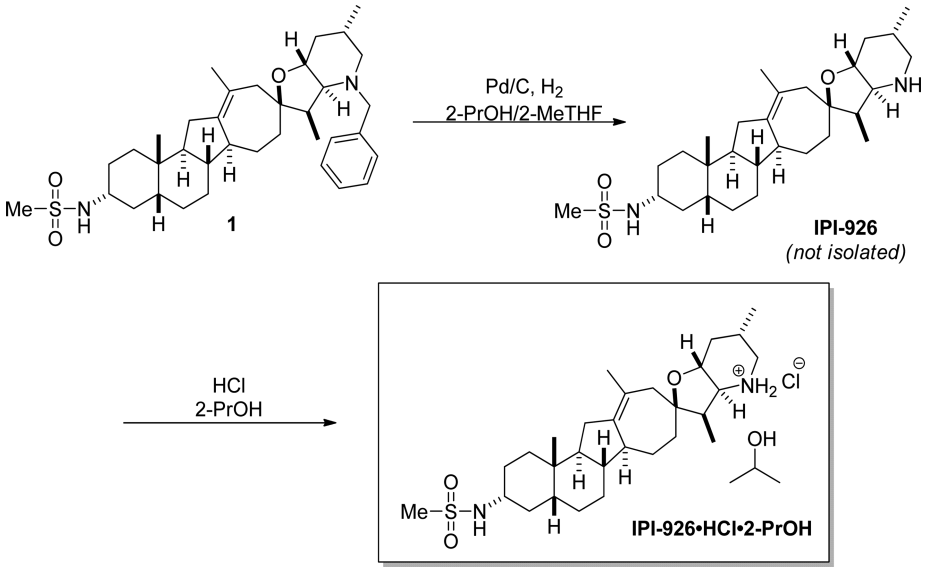


Table 1. Overview of DoE Parameters and Model Terms for Initial Reaction Optimization

parameter varied	response measured	significant terms
Pd loading (0.56, 1.68, 2.80 mol %)	reaction conversion (% area IPI-926; measured values: 12.3−100%)	temperature ($p < 0.0001$)
hydrogen pressure (1, 3, 5 bar)		Pd loading ($p < 0.0001$)
reaction temperature (20, 40, 60 °C)		Pd loading × Pd loading ($p = 0.0207$)
substrate concentration (5, 10% g/mL)		substrate concentration ($p = 0.0096$)

1; Figure 1), with the combination of high catalyst loading (2.8 mol %) and high temperature (60 °C) resulting in complete conversion after 1 h.

The biggest impurity in the catalytic deprotection of compound **1** was ultimately determined to be the over-reduced product, compound **2** (Scheme 2). The relative configuration of compound **2** in the D-ring was determined by single crystal X-ray diffraction analysis of the 4-bromobenzenesulfonamide derivative of compound **2** (Figure 2). However, this first DoE was not able to take the formation of compound **2** into consideration because this product was not detected by the method used to analyze for reaction conversion and purity of the product.

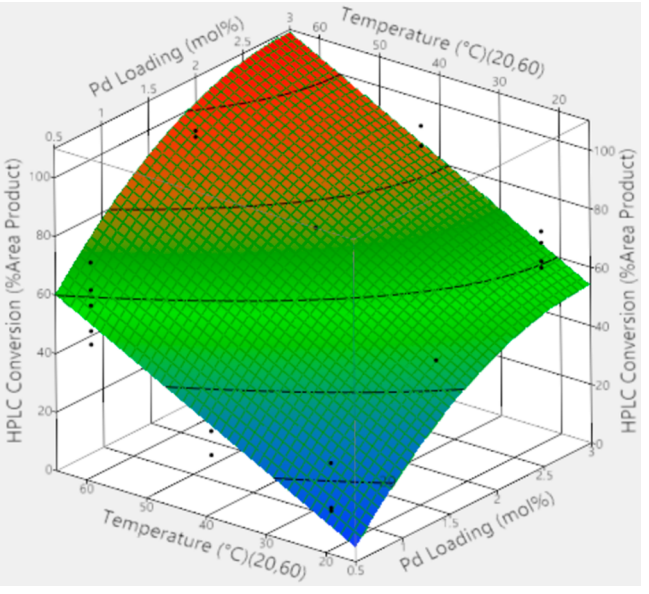


Figure 1. 3D contour plot for reaction conversion as a function of reaction temperature and catalyst loading.[6]

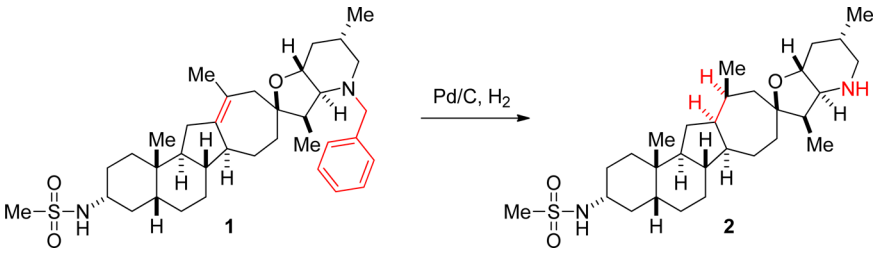
A set of follow-up experiments (eight experiments) looked at the effect of catalyst load (0.84, 1.12, 1.40 mol %) and temperature (20, 40, 60 °C) on the conversion and formation of the olefin-reduced product (compound **2**) after a reaction time of 4 h in a D-optimal design. This side product was tracked by LCMS due to the lack of UV absorbance of the fully reduced product. The experiments confirmed that as reaction conversion increases with increased catalyst loading and temperature, so does the amount of compound **2** that can form (Table 2, Figure 3).

Having completed preliminary screens on this transformation, a more thorough study was initiated. For this investigation, different methods were needed to quantify the reaction conversion and the amount of over-reduction (compound **2**). For this reason, and because reanalysis of the previously generated samples was not feasible, it was not possible to leverage the existing data set and augment the DoE design. Therefore, a new study needed to be initiated. The goal of this investigation was to evaluate the robustness of the current process for the conversion of compound **1** to IPI-926 (Scheme 1) by defining the key parameters[8] and establishing reaction conditions where the transformation can be run with predictable desired output. Specifically, we aimed to understand which parameters influence the formation of compound **2** (Scheme 2) in the transformation and to develop a model to minimize its production.

Based on the previously described studies and historical manufacturing results, four parameters were selected for further optimization as potentially significant for the debenzylation of

DOI: 10.1021/acs.oprd.5b00214
Org. Process Res. Dev. XXXX, XXX, XXX−XXX

Scheme 2. Over-Reduction of Compound 1 To Form Compound 2



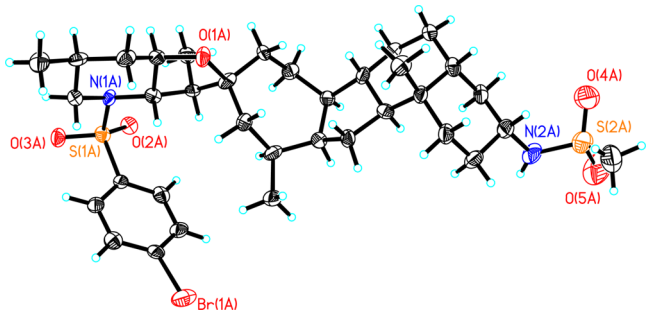


Figure 2. ORTEP plot for the bromobenzenesulfonamide derivative of compound **2**, proving the conformation of the reduced D-ring. Drawing at 50% ellipsoids.

Table 2. Summary of Responses and Model Terms of the Follow-up Debenzylation Study

response measured	significant terms
reaction conversion (% area IPI-926)	temperature (p = 0.0073)
	catalyst load (p = 0.0403)
over-reduction (% area compound **2**)	temperature (p = 0.0005)
	catalyst load (p = 0.0109)

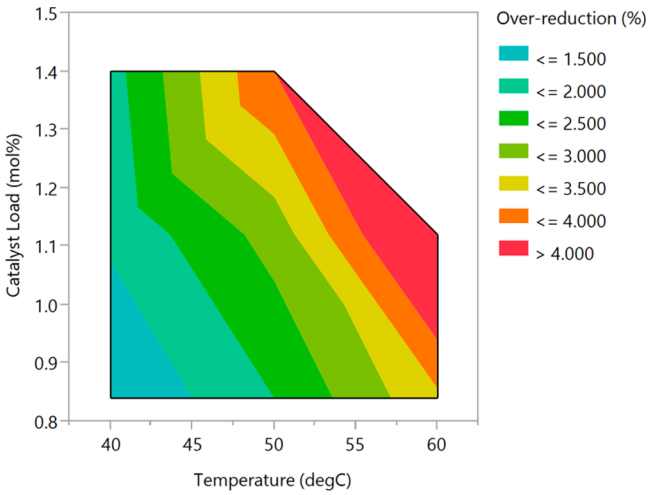


Figure 3. Contour plot for over-reduction (compound **2**) as a function of reaction temperature and catalyst loading for a 4 h reaction hold.[7]

compound **1** and for reduction of the D-ring olefin: (1) reaction time, (2) reaction temperature, (3) catalyst loading, and (4) hydrogen pressure. The range studied for each parameter (Table 3) was then determined to better evaluate the effect of each parameter on the responses and also to predict the optimal conditions. While the reaction concentration was previously determined to have an effect on the reaction performance, this parameter was kept fixed due to process

Table 3. Overview of DoE Parameters and Responses for the Deprotection Reaction Optimization (D-Optimal Design)

parameters studied	parameters held constant	responses measured
reaction time (3–9 h)	reaction solvent: 2-MeTHF (8 vol), 2-PrOH (2 vol)	yield (%, from w/w assay)
temperature (20–60 °C)		conversion (% area IPI-926)
catalyst loading (1–3 mol %)	compound **1** input: 500 mg	over-reduction (% area compound **2**)
H_2 pressure (1–5 bar)		

constraints (i.e., minimum stir volume in reactor as a function of batch size). The DoE study was established to analyze three responses: (1) yield of the deprotection alone (using a HPLC w/w assay), (2) conversion, and (3) over-reduction of compound **1** to compound **2** (using a GC w/w assay).

All experiments were designed to be conducted in a custom-designed pressure reactor (stainless steel) attached to a gas manifold with individual regulators and flash suppressors for each reactor. The system was designed to inert and deliver the desired hydrogen pressure to four independent reactor systems that were stirred independently but heated together in a reaction block. For this reason, experiments were blocked by temperature. In the design of the experiment matrix, the effects of the four variables outlined above were investigated using a D-optimal custom design, in five whole plots for a total of 15 experiments. The design matrix allowed for evaluation of all of the main effects, their quadratic terms, and each cross term. In addition, five additional validation experiments (same scale, same equipment) were added to each whole plot, for a total of 20 experiments (see Supporting Information for data table). The purpose of the validation experiments was to independently confirm that the model generated from the main data set was accurate.

Analysis of the data (see Supporting Information) showed a good correlation (R^2 = 0.98) between reaction conversion and yield up to a level (approximately 99% conversion), after which yield decreased dramatically and the amount of over-reduction increased in similar fashion (Figure 4). This analysis implies that over-reduction is a side reaction whose rate is significantly slower than the desired debenzylation reaction (i.e., compound **2** only forms in appreciable amount after all of compound **1** has been consumed). It further implies that there might be a risk to quality and yield with setting an acceptance criterion for reaction conversion that is too stringent: The cost of getting the last 1% of conversion might far outweigh the benefit.

Of all of the variables, hydrogen pressure had the least pronounced effect on yield and conversion but did have a significant effect (p = 0.0324; see Supporting Information) on the amount of over-reduction that was observed. As such, the optimized conditions proposed by the model, and taking into

DOI: 10.1021/acs.oprd.5b00214
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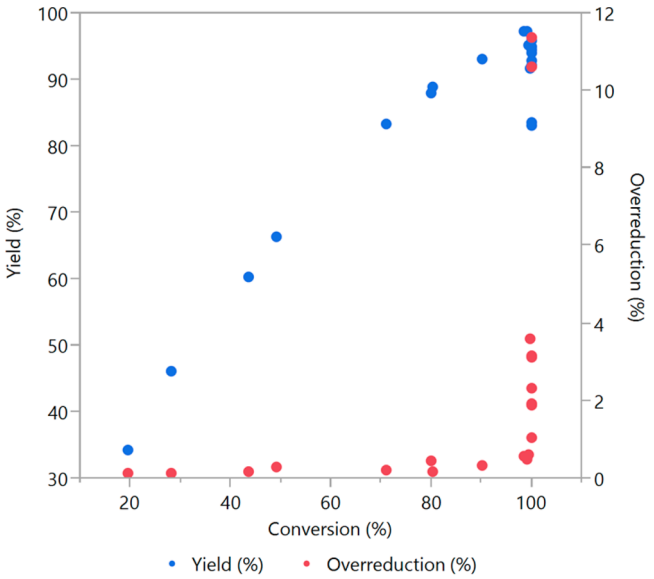

Figure 4. Correlation between reaction conversion and yield (left *y*-axis[9]) as well as reaction conversion and over-reduction (yield of compound **2**; right *y*-axis).[10]

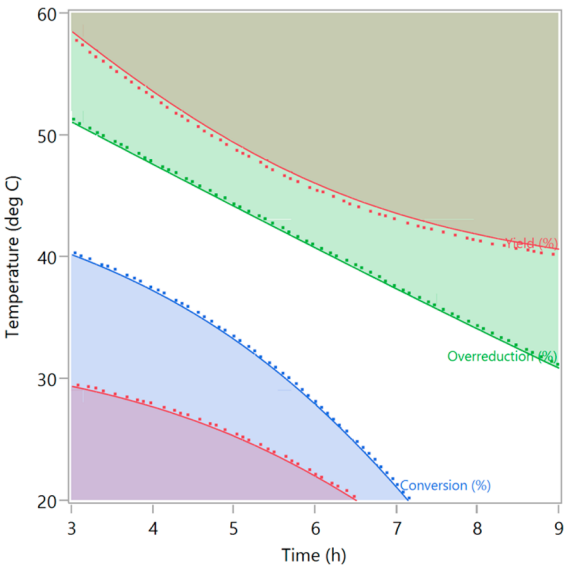

Figure 5. Contour profiler for the reduction of compound **1**.[13]

consideration the consequence of an impacted response,[11] were proposed as follows: (1) 3 mol % catalyst (higher charge); (2) 20−30 °C reaction temperature; (3) 9 h reaction time before first in-process control (IPC) test; (4) 1 bar hydrogen pressure.

Because the constraints of sampling a hydrogenation process for IPC (i.e., exchanging the atmosphere on scale to inert the reactor[12]), the DoE model was applied to determine the optimal sampling point. The key was to minimize the number of samples taken before the reaction was complete, while also ensuring that the reaction would be stopped before a side reaction would take place. In this case, the proposed 9 h reaction hold is based on this empirical model as the time after which the reaction is most likely to be complete, while still having only minimally formed compound **2**. Similarly, while a higher reaction temperature can be used to speed up the reaction, formation of compound **2** becomes an issue much sooner. Based on Figure 5, when the reaction is run at 40 °C, a 3 h (approx.) window exists after a passing IPC and before the formation of compound **2** becomes a problem. On the other hand, if the reaction is performed at 20 °C, that window is much wider (out of scale for the plot).

A contour profiler output of the model is provided in Figure 5. The contour profiler offers a simple graphical representation of the process design space: (1) Yield (red) is affected by both poor conversion and excessive amount of side product; (2) once the conversion (blue) criterion is met, the risk of failing the over-reduction (green) threshold increases. The plot represents the operating window for temperature and time (unshaded region) and shows that, at lower temperatures, a wide design space exists allowing for acceptable conversion with minimization of compound **2** formation.

Summary. A Design of Experiments approach was taken to optimize the debenzylation of compound **1**, looking specifically to minimize side-products and maximize output. The process described required multiple rounds of DoE optimization, primarily due to findings over the course of the study (e.g., formation of compound **2**) which required different analytical techniques. A significant limitation of the approach taken, therefore, was our inability to build on the previous study (i.e., augment the design), which is typically an advantage to using DoE. As much as possible, applications of DoE should make allowances for unexpected results, which would allow for augmenting the studies rather than require a complete redesign.

REMOVAL OF PALLADIUM

Heavy-Metal Scavenging. A scavenger approach[14,15] was taken to remove residual metals from the reaction mixture (compound **1** also contained platinum residue as a result of a previous chemical transformation). Adsorbents on solid support manufactured by PhosphonicS, Silicycle, and Johnson−Matthey were screened along with more common adsorbents and reagents such as $NaHSO_3$ and various types of activated carbon. Sodium bisulfite was ruled out early due to the observed salt formation/precipitation of the product during treatment.

The Silicycle SiliaMetS Triamine scavenger, despite offering initial promising results, afforded unsatisfactory Pd and Pt removal during preliminary scale-up to beyond 40 g. As a result, the Silicycle SiliaMetS Diamine and Thiourea, as well as the PhosphonicS STA3 scavengers were further investigated. The Silicycle SiliaMetS Thiourea scavenger, though effective, was ruled out because of the concern over genotoxicity risk posed by leached thiourea, particularly because this was a final DS-isolation step.[16] The PhosphonicS STA3 scavenger (Figure 6) was ultimately chosen for its superior removal of both platinum and palladium (to <1 ppm in lab-scale runs) and benign ligand profile.

After removal of the bulk catalyst following reaction completion, the resulting solution is treated with PhosphonicS STA3 (1 wt relative to the compound **1** charge) at 50 ± 5 °C for 18 h. The bulk of the adsorbent is then removed by filtration, and the filtrate is clarified through a 0.2−0.45 μm

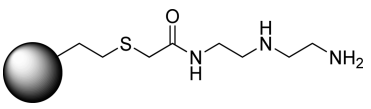

Figure 6. Functional group of the PhosphonicS STA3 scavenger.

DOI: 10.1021/acs.oprd.5b00214
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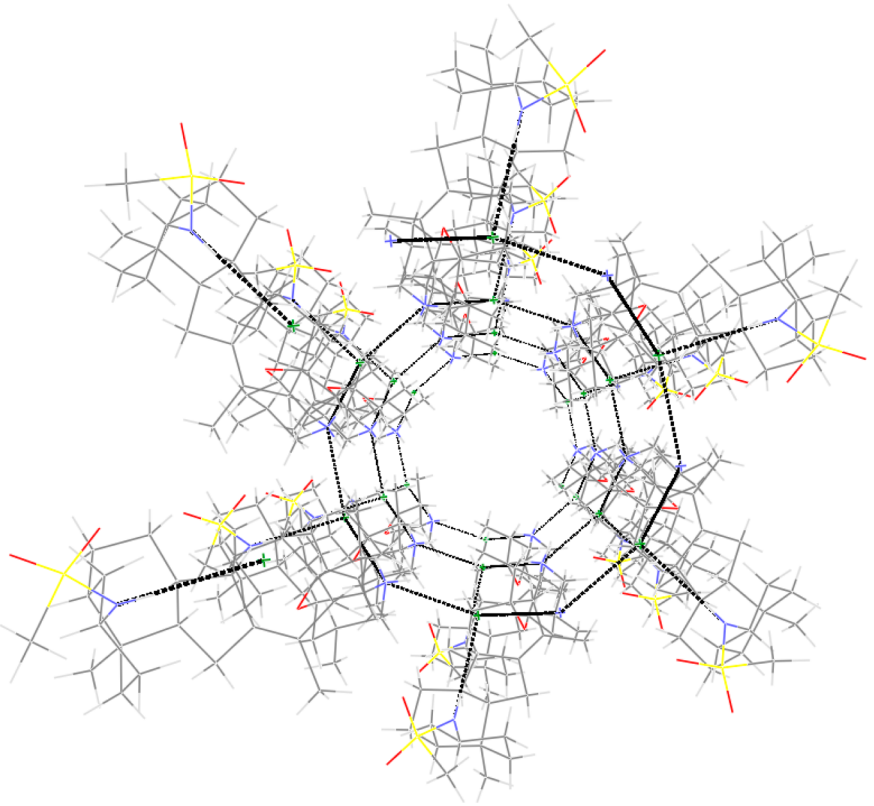

Figure 7. Crystal structure of IPI-926·HCl: Perspective view down the hexagonal tunnels in IPI-926. Hydrogen bonding is shown as dashed lines.

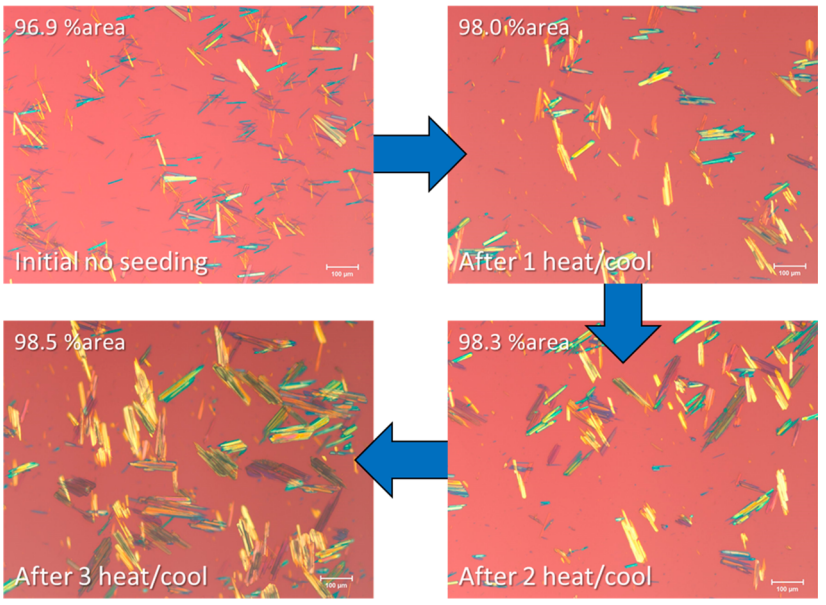

Figure 8. Illustration by PLM of the effect of heat/cool cycles on the purity (top left in each image) and crystal size of isolated IPI-926·HCl from the initial salting procedure.[19]

filter medium. The resulting solution is transitioned to the salting and crystallization stage via solvent exchange.

SALT FORMATION

IPI-926 Salt Screen and HCl Polymorph Screen. As a result of a salt and polymorph screen for IPI-926, 13 potential salts were obtained with eight (8) different counterions (acetic acid, ascorbic acid, benzoic acid, fumaric acid, glycolic acid, hydrochloric acid, maleic acid, and sulfuric acid), in addition to the crystalline free base, amorphous material, and counterion/IPI-926 heterogeneous mixtures. Eight (8) salt forms remained stable after a stability study, and three (3) additional, new salt forms crystallized under stress conditions. Out of nine (9) salt forms further characterized by thermal analyses, all were hydrates, solvates, and mixed hydrates/solvates.

A crystal structure of the most stable polymorph of the HCl salt of IPI-926 was obtained (Figure 7). Based on the crystal structure, IPI-926 molecules are shown to be linked by

DOI: 10.1021/acs.oprd.5b00214
Org. Process Res. Dev. XXXX, XXX, XXX−XXX

hydrogen-bonding through the chloride ion, creating hexagonal tunnels down the *c*-axis capable of being occupied by solvent molecules, typically 2-PrOH, which is the solvent from which this salt is isolated. In a practical sense, this means that the isolated IPI-926 drug substance will therefore contain levels of 2-PrOH that are significantly higher than ICH limits of 5000 ppm:[17,18] The theoretical amount of 2-PrOH for the stoichiometric solvate would be 10% or 100 000 ppm, and typical values observed ranged from 80 000 ppm to 100 000 ppm. However, these levels were deemed acceptable for this drug substance, as the drug product manufacture employed a low shear wet granulation, which displaced the 2-PrOH with water. The resulting drug product contained typically 50−200 ppm of 2-PrOH.

IPI-926·HCl Salt Formation and Isolation. The original conditions used for the synthesis of IPI-926·HCl called for removing and salting 1.5% of the batch for use as a seed in the main batch. The seed was added after an initial addition of the HCl to the batch (prior to nucleation), and then the remaining HCl solution (in 2-PrOH) was added over the course of 7−8 h. This approach addressed the question of an appropriate seed for manufacturing under cGMP (i.e., whether a seed needs to be generated under cGMP or can be treated as a raw material with the proper release controls) and took a conservative approach to dealing with this issue. Moreover, since the impact of seed type (i.e., crystal size, habit, etc.) had not been established, this approach allowed for the most flexibility during production. However, upon review of this process, a number of limitations were identified in this salting procedure: (1) the quantity of the seed was small, and set arbitrarily (without understanding of the IPI-926·HCl solubility); (2) the addition rate of the HCl was very slow (took a very long time in production); and (3) the procedure as a whole was very complicated operationally. Additionally, the stoichiometry of the HCl used for this salting had been arbitrarily set to 1.3 equiv relative to the starting material, compound **1**, with no clear understanding of the effect of excess HCl on the purity of the isolated material or on the actual yield of the debenzylation reaction. For these reasons, and in an effort to produce material that was of consistently high purity, the procedure was revised.

Second Generation Investigation. After a number of experiments probing the effect of temperature, HCl equivalents, seed quantity, seed type (added as a solid or as a freshly prepared suspension), and addition rate, the highest purity material was obtained after multiple heat/cool cycles of the IPI-926·HCl crystals in 2-PrOH (Figure 8). Additional experiments demonstrated the benefit of the seed crystals in terms of allowing for a controlled crystal growth rather than a sudden precipitation of the salt due to improper control of supersaturated IPI-926·HCl (data not shown). Combined, these results suggested that both the addition of a heat/cool cycle and the use of a seeding protocol would improve the quality of the product.

The behavior of the IPI-926·HCl crystals in suspension was investigated at room temperature and during reflux using in-line tools such as focused beam reflectance measurement (FBRM) and particle vision and measurement (PVM) and off-line tools such as polarized light microscopy (PLM). It was hypothesized that the heat cycle broke up large agglomerations resulting from uncontrolled nucleation/growth that could trap impurities (initial nucleation results in acicular crystals) and subsequently led to secondary crystal growth (as suggested by the appearance of the stacked crystals in Figure 8) through dissolution of the smaller particles and regrowth from existing crystals. The result of the heating cycle was therefore a product with higher purity and a more uniform particle size that ultimately offered an improved filterability/processability. The length of the heat cycle was probed but no adverse consequence (yield or purity of the isolated solid) was observed from heating the suspension at reflux for up to 35 h. Specifically, the formation of 2-chloropropane[20] was investigated, and no clear effect on the content of this impurity in the isolated DS was observed from this heat cycle. Should 2-chloropropane be forming as a result of this reaction, it appears to neither be trapped in the isolated drug substance nor result in formation of other impurities, including the *N*-alkylation product, in appreciable levels.

Since the debenzylation reaction was studied separately from the salt formation, the two processes had to be decoupled.[21] In order to do so and to normalize the equivalents of HCl used for the salting and crystallization of IPI-926, it became important to assay the amount of IPI-926 in solution prior to the crystallization and to properly titer the HCl equivalents used for the crystallization of IPI-926·HCl. Operationally, since the IPI-926 solution obtained after the scavenging operation is in a mixture of 2-MeTHF and 2-PrOH, a distillation is performed to remove water and exchange the solvent to mostly 2-PrOH prior to the salting. The effect of 2-MeTHF on the yield of the salting and purity of IPI-926 was investigated. To this end, no significant difference in purity or yield was observed for any of the doped samples ranging from 0 to 20 000 ppm residual 2-MeTHF. Typical 2-MeTHF content in the 2-PrOH solution of IPI-926 used in the crystallization was shown to be <100 ppm. The assay of the IPI-926 solution is therefore performed on the 2-PrOH solution prior to salting.

Salting DoE. Finally, a DoE approach was taken to fully understand the performance of the IPI-926 salting procedure. The amount of seed used was increased from 1.5% and fixed at 5% based on the solubility[22] of IPI-926·HCl in 2-PrOH and to ensure that the seed would not dissolve completely in the reaction mixture. An I-Optimal design was chosen to investigate the following variables: (1) the maximum cycle temperature (the length of the cycle was fixed at 6 h; 25 °C, 55 °C, ∼84 °C (reflux)), (2) the equivalents of HCl relative to IPI-926 (1.0, 1.15, 1.3 equiv), (3) the addition rate of HCl (fast, moderate, slow; corresponding to an addition time of 0, 1, and 2 h, respectively[23]), and (4) the final aging temperature (0 °C, 12.5 °C, 25 °C). These variables were combined into a custom I-optimal design and resulted in a study design comprising 16 experiments. The responses monitored were (1) product yield, (2) purity, and (3) mass balance including the mother liquors (a representation of any decomposition or side-reaction that could have occurred). All of the study was completed from a single lot of IPI-926 solution in 2-PrOH, which was partitioned and assayed after distillation to ensure a uniform starting material for all salting experiments.

Yield was most affected by the stoichiometry of the HCl added (Figure 9), but only for the low charge, which implies that a slight excess of HCl is necessary to ensure maximum recovery. The purity was most affected by the rate of HCl addition (Figure 10), with the most pronounced effect being observed when the addition rate was slowed down from "fast" to "moderate". The lower purity can be explained by the fact that a faster addition rate of HCl will result in an uncontrolled crystallization of the HCl salt and likely result in trapping of impurities during the salt formation. Finally, mass balance was also affected by the rate of HCl addition (Figure 11). This last

DOI: 10.1021/acs.oprd.5b00214
Org. Process Res. Dev. XXXX, XXX, XXX−XXX

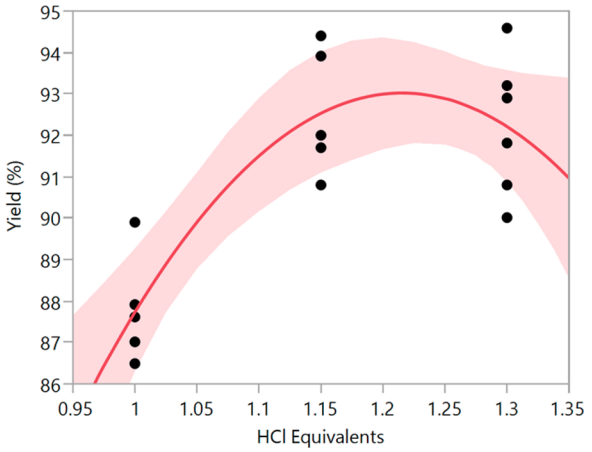

Figure 9. Effect of HCl equivalents on the yield of the IPI-926·HCl salt formation.[24]

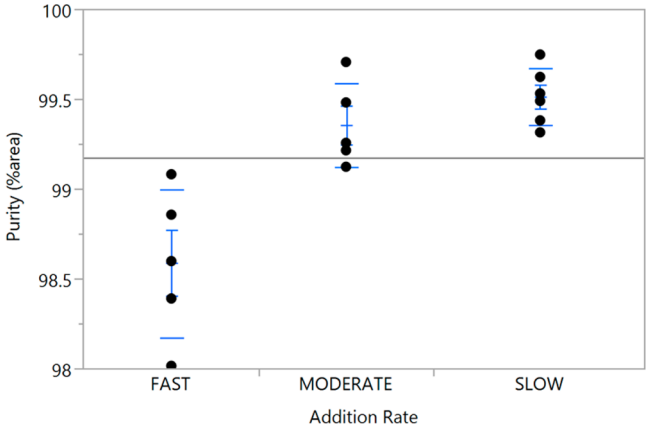

Figure 10. Effect of HCl addition rate on the purity of the isolated IPI-926·HCl salt.[25]

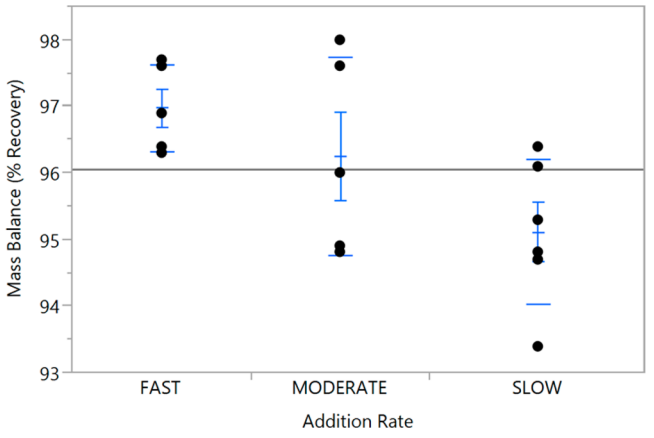

Figure 11. Effect of HCl addition rate on the total mass balance of IPI-926.[26]

observation suggested that a stability issue might arise, whereby a prolonged exposure to the reaction conditions (i.e., through a slow HCl addition) could result in impurity formation.

The salting procedure was effective at purging reaction side-products and starting material but resulted in few new impurities. The over-reduced side product compound **2** (Scheme 2) was removed efficiently in the process of salting, and no single impurity >0.2% area was observed in lots of IPI-926·HCl manufactured using this process. The penultimate, compound **1**, was also significantly removed during the salting. However, two new impurities at RRT 1.10 and 1.11 were generated in the process of producing IPI-926·HCl. Both of these impurities have an m/z of 505 using electrospray ionization in the positive mode (ESI+), which is the same molecular ion as IPI-926, suggesting an isomer of IPI-926. Internal work identified the structure of the RRT 1.10 impurity via isolation and modification as the phenylurea analogue (Figure 12). The relative configuration of the impurity was determined using single crystal X-ray diffraction. The structure of the RRT 1.11 impurity could not be conclusively established. Nevertheless, in all cases where the mass balance was low (e.g., Figure 11), higher levels of the RRT 1.10 and RRT 1.11 impurities were observed in the solid, the mother liquor, or both (but primarily in the mother liquor).

From the data generated, the optimal conditions (based on yield, purity, and mass balance; Figure 13) for the salt formation were determined to be as follows: 1.2 equiv of HCl; a heat cycle to reflux (85 °C); and a moderate or slow addition rate (~1−2 h/batch). Analysis showed that the final (aging) temperature did not have a statistically significant effect on any of the measured outputs. Separately, the source of the seed (i.e., whether it is freshly prepared or from a previous batch) was shown to have no effect on the habit or purity of the isolated solids. With these optimal conditions, the formation of the RRT 1.10 and 1.11 impurities is kept to a minimum, and they have typically not been observed in production.

In addition to providing the measured responses above, the DoE-optimized salting conditions offered insight into control over the habit of the isolated crystals (Figure 14) as well as their analytical behavior when analyzed by differential scanning calorimetry (DSC). When HCl was added quickly, large prism-like crystals were obtained, and the isolated solid had two DSC endotherms in the 210−240 °C range. This behavior is likely due to differential growth kinetics attributable to the large supersaturation of the IPI-926·HCl salt created under these conditions. However, when HCl was added slowly, needle-like crystals were obtained, and the solids had only one DSC endotherm in the 220−240 °C range. Although the number of DSC events/endotherms were not an indication and have not been tied to polymorphs of IPI-926·HCl, we found it more desirable to systematically generate material with a single DSC endotherm in the 220−240 °C range.[28] In addition to this observation, crystals that were needles were found to have a systematically higher purity (Figure 15).

CONCLUSIONS

A design of experiments approach, through iterations of the different process operations, was applied to the development and optimization of a deprotection reaction and salt formation to generate IPI-926·HCl. The development has enabled a robust process capable of generating product with reasonable yields and high purities suitable for further development. The resulting process was applied to production of IPI-926 Drug Substance at kilogram scale (up to 15 kg input of compound **1**) and was successful through multiple batches of production. The approach taken to enable a process from early development through manufacture highlights the strength of DoE through multiple stages of development.

DOI: 10.1021/acs.oprd.5b00214
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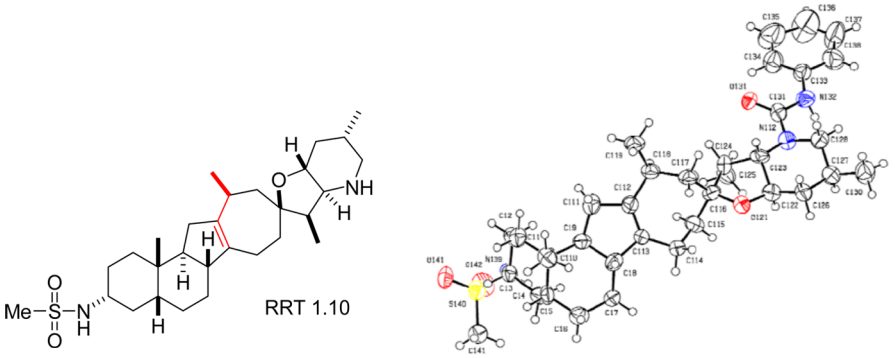

Figure 12. Structure of the RRT 1.10 impurity and ORTEP plot of its urea derivative.

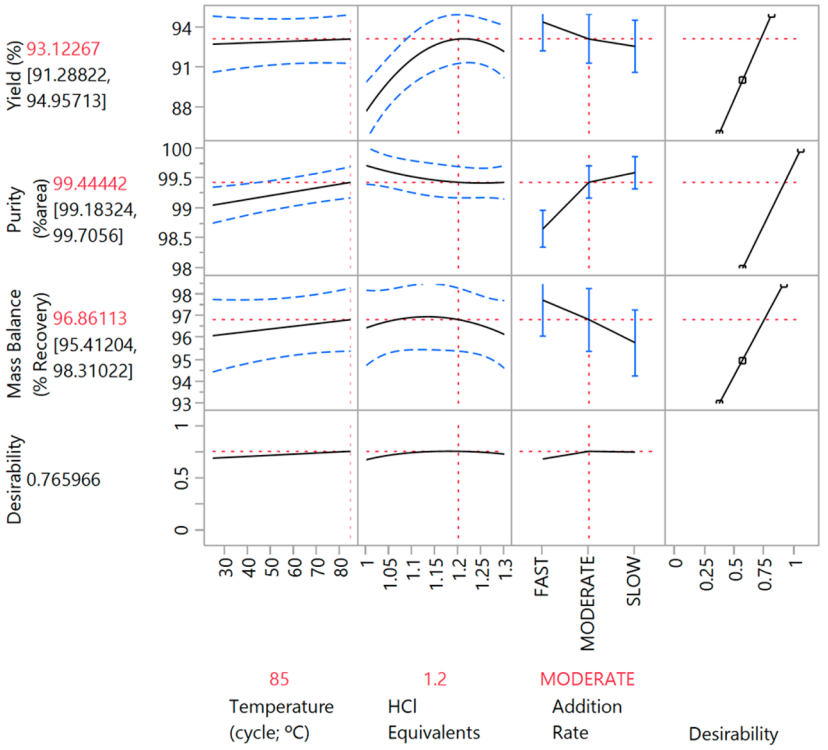

Figure 13. Prediction profiler for the salting of IPI-926 based on the DoE results.[27]

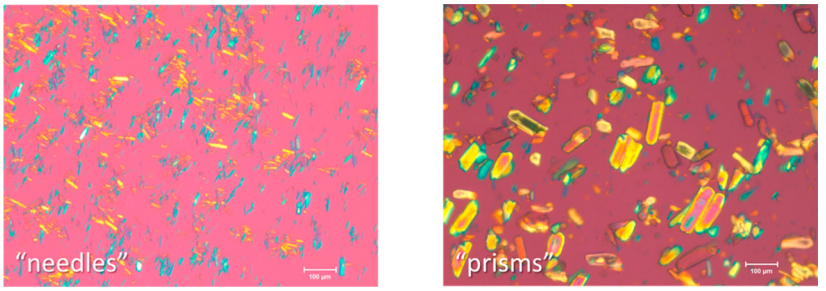

Figure 14. Example of two types of crystal habits of IPI-926·HCl observed. Both correspond to the same polymorph.[29]

EXPERIMENTAL PROCEDURE

General Methods. All experiments involving water-sensitive compounds were carried out under argon or nitrogen and scrupulously dry conditions, using commercially available anhydrous solvents. Electrospray positive ionization mass spectrometry was performed on samples using a reversed phase HPLC-MS system. The system was equipped with a diode array UV detector and a single quadrupole mass spectrometer and utilized a reversed-phase HPLC gradient method.

Preparation of IPI-926·HCl. To an inerted reactor, compound **1** was charged (14.96 kg, 25.1 mol) followed by 2-MeTHF (105 L; 7 vol), and the resulting solution was stirred at 25 ± 5 °C. To a separate reactor, 5% Pd/C, Johnson-

DOI: 10.1021/acs.oprd.5b00214
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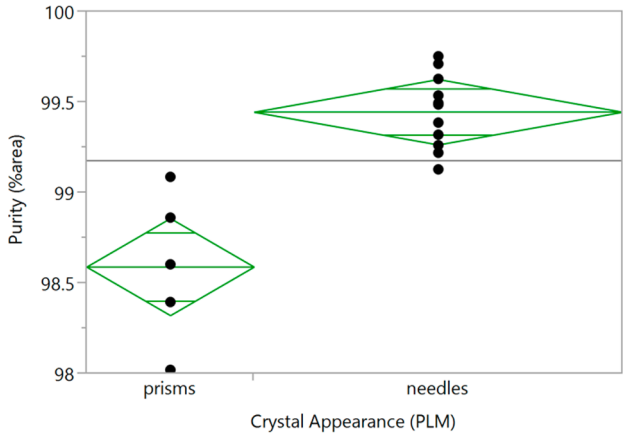

Figure 15. Correlation between crystal appearance (as established by PLM) and the purity of the isolated material.[30]

Matthey type A405032-5 (3.98 kg; 4.94% assay; 58.9% water) was charged. After inerting the reactor using nitrogen or argon, the solution of compound **1** in 2-MeTHF was charged to the reactor containing the catalyst. The first reactor was rinsed with 2-MeTHF (15 L) followed by 2-PrOH (30 L) and the rinse was added to the solution containing the starting material and catalyst. The reactor was pressure-tested, then inerted using nitrogen or argon once more. After adjusting the batch temperature to 25 ± 5 °C, the atmosphere in the reactor was exchanged for hydrogen (0.5 bar), and the reaction was allowed to proceed for a minimum of 5 h. Reaction conversion was determined by RP-HPLC, inerting the reaction atmosphere with nitrogen each time a sample was taken. Upon complete reaction (12 h[31]), the reactor was inerted, and the bulk catalyst was removed by filtration (ZetaPlus 30SP) using nitrogen pressure. The reactor, filter, and ancillary lines were rinsed with a mixture of 2-MeTHF (72 L, 4.8 vol) and 2-PrOH (18 L, 1.2 vol), combining the filtrate and wash into a reactor that was previously charged with the Phosphonics STA3 scavenger (12.3 kg; 0.82 wt; washed with 2-MeTHF and 2-PrOH prior to use). The mixture was heated to 50 ± 5 °C, and the batch temperature was maintained for 18−24 h. The batch was cooled to 20 ± 5 °C, and the scavenger was removed by filtration using nitrogen pressure. The cake was rinsed with a mixture of 2-MeTHF (20 L; 1.34 vol) and 2-PrOH (5 L; 0.33 vol). The filtrate and wash were combined into a clean reactor and tested for residual Pd and Pt (Actual: Pd < 0.5 ppm; Pt 1.5 ppm). The batch was concentrated under reduced pressure to a residual volume of 89 L (5.93 vol), and 2-PrOH (165 L; 11 vol) was charged to the batch. The distillation and charge were repeated two more times, after which an additional distillation was completed, and 2-PrOH (38 L; 2.54 vol) was charged. After cooling the batch to 20 ± 5 °C, the residual water and 2-MeTHF was determined using an NIR probe (FIO: 0.086% w/w water; 0.032% w/w 2-MeTHF). The weight of the batch was measured (103.8 kg), and its content of IPI-926 was determined using HPLC assay (10.5% w/w), which corresponds to a yield of 10.9 kg of IPI-926 or 85.9% from compound **1**. The solution was polish-filtered into a clean reactor, and the volume of the solution was adjusted with 2-PrOH (78 L) to correspond to 19 L per kg of IPI-926. The batch temperature was adjusted to 25 ± 5 °C, and IPI-926·HCl seed (0.654 kg; 0.06 wt relative to IPI-926 FB) was charged. To the batch, a solution of HCl in 2-PrOH (20.2 L of 1.25 M; 1.20 equiv; charge is based on CoA titer) was dosed over a period of 2−2.5 h, maintaining a batch temperature of 25 ± 5 °C throughout the addition. After completion of the addition, the batch was aged for 30−60 min, then heated to reflux for 6−12 h, cooled back down to 25 ± 5 °C, and held at that temperature for 5−10 h. The batch was isolated on an agitated filter dryer using nitrogen pressure, and the cake was washed with 2-PrOH (22 L, 1.5 vol) twice. The product was dried at a jacket temperature of 45 °C until an LOD <2.30% (w/w) was achieved. Yield: 11.5 kg (73% from compound **1**, correcting for the seed). HPLC purity: 99.9% area (compound **2** content: 0.08% w/w). Assay: 83.7% w/w (as-is), 99.1% w/w (anhydrous, solvent-free). Moisture content: 1.6% w/w. Chlorine content: 5.72% w/w. Residual solvents: acetone (720 ppm); acetonitrile (<41 ppm); 2-MeTHF (none detected); 2-propanol (81 147 ppm); toluene (<90 ppm). Residual metals: palladium (0 ppm); platinum (0 ppm); ruthenium (0 ppm). Additional data for the IPI-926 free base: ^{1}H NMR (400 MHz, $CDCl_3$) 6.90 (br s, 1H), 3.31 (dt, J = 10.6, 3.8 Hz, 1H), 3.20 (br s, 1H), 3.10 (dd, J = 13.7, 4.5 Hz, 1H), 2.91 (s, 3H), 2.62 (dd, J = 9.9, 7.6 Hz, 1H), 2.33 (br d, J = 14.5 Hz, 1H), 2.27−2.15 (m, 1H), 2.10 (dd, J = 14.5, 6.9 Hz, 1H), 1.99−1.17 (m, 28H), 1.05 (q, J = 11.6 Hz, 1H), 0.93 (d, J = 7.4 Hz, 3H), 0.88 (d, J = 6.6 Hz, 3H), 0.86 (s, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) 140.47, 124.53, 82.48, 76.97, 63.73, 54.08, 53.87, 50.12, 49.98, 47.19, 44.73, 42.27, 42.10, 40.24, 37.55, 37.44, 36.04, 34.44, 31.87, 31.33, 30.46, 29.79, 28.37, 27.94, 26.26, 24.19, 22.70, 18.92, 10.19 ppm; MS: m/z = 505.29 $[M + H]^+$.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.oprd.5b00214.

Data tables from the DoE studies (PDF)

Single crystal structure of the bromobenzenesulfonamide derivative of compound **2** (CIF)

Single crystal structure of IPI-926·HCl (CIF)

Single crystal structure of the urea derivative of the RRT 1.10 impurity (CIF)

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

2-MeTHF, 2-methyltetrahydrofuran; 2-PrOH, 2-propanol; DoE, design of experiments; DS, drug substance; DSC, differential scanning calorimeter; EtOH, ethanol; FB, free base; FBRM, focused-beam reflectance measurement; HPLC, high performance liquid chromatography; IPC, in-process control; LCMS, liquid chromatography/mass spectrometry; LOD, loss on drying; ND, not detected; NIR, near infra-red; NMR, nuclear magnetic resonance; PLM, polarized-light microscopy; PVM, particle vision and measurement; RP, reverse phase; RRT, relative retention time; QbD, quality by design

DOI: 10.1021/acs.oprd.5b00214
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REFERENCES

(1) Tremblay, M. R.; Lescarbeau, A.; Grogan, M. J.; Tan, E.; Lin, G.; Austad, B. C.; Yu, L.-C.; Behnke, M. L.; Nair, S. J.; Hagel, M.; White, K.; Conley, J.; Manna, J. D.; Alvarez-Diez, T. M.; Hoyt, J.; Woodward, C. N.; Sydor, J. R.; Pink, M.; MacDougall, J.; Campbell, M. J.; Cushing, J.; Ferguson, J.; Curtis, M. S.; McGovern, K.; Read, M. A.; Palombella, V. J.; Adams, J.; Castro, A. C. *J. Med. Chem.* **2009**, *52*, 4400−4418.

(2) Goos, P.; Jones, B. *Optimal Design of Experiments: A Case Study Approach*; John Wiley & Sons, Ltd.: New York, 2011.

(3) 5% Pd/C, nominally 50% water wet.

(4) JMP (SAS Institute, Inc.) was used to create all DoE models. Version 11.1.1 was used for all outputs. Models were refined and optimized using a Standard Least Squares approach, to eliminate terms that did not contribute significantly to the model ($p > 0.05$, starting with the highest values) or using a Stepwise approach, with the same criterion. All data tables and analyses are available in the Supporting Information section.

(5) In this initial screen, it was easier to monitor for consumption of starting material because at this stage of development, a complete understanding of reaction side products was not available. Therefore, by going with a 1 h reaction time, we were better positioned to make a call on what conditions made an impact. With a longer reaction hold (e.g., 5 h), it was expected that all reactions would be complete, so the analysis would have needed to rely more heavily on a impurity profile that was not well understood.

(6) Data points (black) are overlaid on the calculated surface (blue to red gradient with reaction conversion contours), confirming the accuracy of the model generated.

(7) These data confirm that with increased temperature and catalyst, the amount of over-reduction also increases.

(8) At this stage of development, the Critical Quality Attributes of IPI-926 were not finalized. While the parameters investigated could ultimately be considered critical following a careful risk analysis, that was not necessarily the aim of this study.

(9) $R^2 = 0.98$ when conversion < 99%.

(10) This relationship demonstrates that over-reduction only becomes significant once > 99% conversion is observed.

(11) For example, a poor conversion is easily remedied in practice for this reaction by additional reaction time, while there is no remedy for the formation of compound **2**.

(12) An application of Process Analytical Technology (PAT) to this problem could remedy the issue. For example, hydrogen uptake or a ReactIR signal could be used to monitor reaction conversion in the lab or in the plant. However, neither of these were available to us at the time to develop in lab studies. While plant operations did monitor hydrogen uptake (g/h), that metric was not applied as an IPC.

(13) The contours are set as follows: yield (red) shaded < 95%; conversion (blue) shaded < 95%; over-reduction (green) shaded > 1%. The unshaded region corresponds to the "Design Space" where the above criteria will be met for a catalyst load of 3 mol% and hydrogen pressure of 1 bar. If catalyst load and/or hydrogen pressure are varied, then the design space will also vary.

(14) Galaffu, N.; Man, S. P.; Wilkes, R. D.; Wilson, J. R. H. *Org. Process Res. Dev.* **2007**, *11*, 406−413.

(15) Barbaras, D.; Brozio, J.; Johannsen, I.; Allmendinger, T. *Org. Process Res. Dev.* **2009**, *13*, 1068−1079.

(16) Rodriguez-Arnaiz, R. *Mutat. Res., Genet. Toxicol. Environ. Mutagen.* **1997**, *395*, 229−242.

(17) ICH. Guidance for Industry, Q3C Impurities: Residual Solvents. FDA, Ed. 1997.

(18) ICH. Guidance for Industry, Q3C—Tables and List (Revision 2). FDA, Ed. 2012.

(19) Magnification is 100×; the scale bar is 100 μm.

(20) Snodin, D. J. *Org. Process Res. Dev.* **2010**, *14*, 960−976.

(21) It is understood that the two reactions could have been studied together as a single DoE. In this case, since it was believed that effects from the debenzylation would not necessarily have an impact on the salt formation, the two were studied independently.

(22) The solubility of IPI-926·HCl was measured in 2-PrOH at variable temperatures using a Crystal16.

(23) A 0 h addition time is not feasible under most circumstances, either in a lab setting, or in production. The intention in this design feature of the DoE was not to implement this parameter in production. Instead, this addition time was chosen to exaggerate any effect that could be seen from an addition perspective.

(24) The data are fit with a quadratic function, and the shaded region represents the 95% confidence interval for the fit. (R^2 Adj = 0.66; ANOVA Prob > |*F*| 0.0004*).

(25) Addition rate is defined by total addition time of the HCl: fast = "0" h; moderate = 1 h; slow = 2 h.

(26) The mass balance corresponds to the isolated yield of IPI-926 combined with the residual IPI-926 in the mother liquor. Addition rate is defined by total addition time of the HCl: fast = "0" h; moderate = 1 h; slow = 2 h.

(27) Desirability plots (right column) aims to maximize the individual outputs and result in individual desirabilities (bottom row) for each response. Overall desirability is optimized as a function of the desirability each individual output (e.g., maximize yield).

(28) The DSC behavior of IPI-926 was not fully understood, but the single endotherm was viewed qualitatively as an indication of a more robust manufacturing process.

(29) Magnification is 100×; the scale bar is 100 μm.

(30) ANOVA $p < 0.0001$*. Means for one-way ANOVA: prisms: 98.59% area; needles: 99.44% area.

(31) Reaction progress was as follows: 5 h 20 min (24.1% area residual SM); 8 h (7.0% area residual SM); 10 h (1.7% area residual SM); 12 h (ND residual SM).

DOI: 10.1021/acs.oprd.5b00214
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