Organic Process Research & Development

Review

pubs.acs.org/OPRD

Design of Experiments (DoE) and Process Optimization. A Review of Recent Publications

Steven A. Weissman*,† and Neal G. Anderson*,‡

†J-Star Research, 3001 Hadley Road, Unit 1, South Plainfield, New Jersey 07080, United States

‡Anderson's Process Solutions, 7400 Griffin Lane, Jacksonville, Oregon 97530, United States

Supporting Information

ABSTRACT: Statistical design of experiments (DoE) is a powerful tool for optimizing processes, and it has been used in many stages of API development. This review summarizes selected publications from *Organic Process Research & Development* using DoE to show how processes can be optimized efficiently and how DoE findings may be applied to scale-up.

INTRODUCTION

Statistical design of experiments (DoE) is a powerful approach to optimizing chemical processes.[1] In 2002 the editor of this journal expressed the opinion that DoE deserved to be taught in academia and should be used more often in industry.[2] Over the past 11 years the application of DoE to pharmaceutical process development[3] has greatly increased,[4] as measured by the volume of DoE-related publications in OPRD (Figure 1). For the period 2003−2007, the average was 6.0 publications/year, but that figure jumped to 14.7 publications/year for the period 2008−2013, a 145% increase.

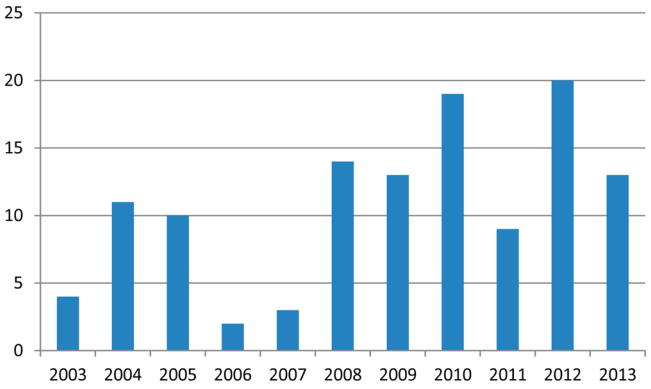


Figure 1. Number of DoE-related publications in OPRD annually from 2003−2013.

Some of the reasons for this upward trend in DoE implementation are as follows:

1. Changing R&D Environment: The pressure of compressed API development timelines is always increasing.[5] This, combined with recent reductions in the pharmaceutical work force, means process scientists are being asked to do more with fewer resources. Also, several new FDA designations are aimed at providing expedited reviews of drugs serving unmet medical needs.[6] Accordingly, efficiency becomes paramount and DoE is a tool to resolve optimization issues in a timely manner.

2. Technological Advances: The once daunting prospect of setting up the multiple reactions required by a DoE has diminished with advances in parallel reactors and analytical assays/instrumentation. Setting up 19 reactions is no longer considered to be the barrier it was even 15 years ago. High-throughput screening (HTS) tools and flow chemistry setups have been widely adopted.

3. Changing Drug Substance Regulatory Environment: The unmistakable trend away from the traditional quality by testing and toward quality by design (QbD) has led to increased awareness of the concept of design space.[7] DoE, as part of process validation, plays a central role defining the acceptable ranges for the critical process parameters. The FDA now *expects* DoE to be part of the NDA submissions.[8]

4. Increased Implementation of Green Chemistry Principles: Several of the well-known 12 Green Chemistry Principles advocate for using lesser amounts of solvents and reagents and for increased process efficiency as a means toward generating less chemical waste. Accordingly, manufacturers are increasingly implementing programs[9] and applying metrics[10] to assess the green chemistry performance of their processes. DoE's ability to enable scientists to hone in on the optimal reaction conditions has helped it gain wider acceptance.

Clearly the perceived barriers[11] to implementing DoE in the process chemistry setting are being overcome, leading to wider adoption of this powerful tool.

The traditional optimization approach, varying one factor/variable at a time (OFAT, also called OVAT),[12] suffers from many drawbacks. First, it rarely uncovers the optimal conditions, in large part due to the fact that the outcomes are highly dependent on the starting point. Second, the OFAT approach is unable to separate the "noise" (the inherent run-to-run variation of a system) of a reaction from actual improvement unless a significant number of reactions are repeated using the same conditions. The systematic approach inherent in DoE eliminates researcher bias and often will lead to reaction conditions that one had not considered previously. But the most significant

Special Issue: Application of Design of Experiments to Process Development

Received: May 28, 2014
Published: June 27, 2014

   dx.doi.org/10.1021/op500169m | *Org. Process Res. Dev.* 2015, 19, 1605−1633

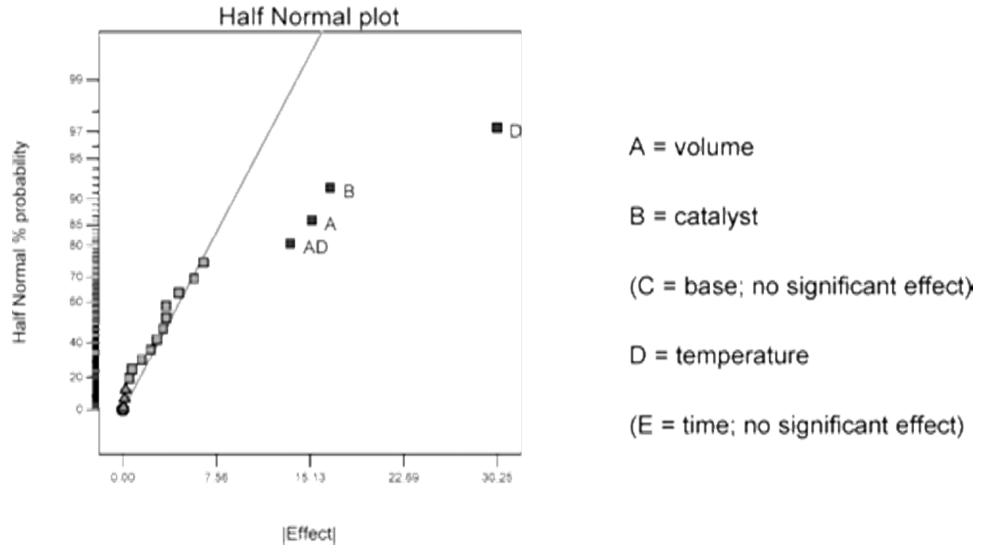


Figure 2. Half-normal plot showing the influence of volume, catalyst charge, and temperature on a Heck reaction yield. Volume and temperature interact (AD) to influence the yield. The farther that data points are from the diagonal line, the greater the influence of the factor. Reprinted with permission from Aggarwal, V. K.; Staubitz, A. C.; Owen, M. *Org. Process Res. Dev.* **2006**, *10*, 64. Copyright 2006 American Chemical Society.

advantage to using DoE is the ability to quickly detect how interactions between factors can affect product yield and quality.

Also, by simultaneously varying parameters, the DoE approach can be more efficient than that achieved by the traditional approach of varying one factor/variable at a time. For instance, if the OFAT approach was used to investigate the influence of three factors on a reaction (temperature, concentration, and reagent stoichiometry), eight experiments would be required; but more information could be generated through four experiments in a half-factorial DoE. Running a DoE may seem daunting initially, since the number of experiments to be run is defined at the beginning, unlike the traditional approach. But even if the effort to perform the multiple reactions and assays engendered by a DoE is time-consuming, the *quality* and thoroughness of the information obtained outweigh the effort.

Generally there are three times in the life of a process when DoE may be used: early in process development when the best conditions are not obvious; when exploring robustness to assist in defining a design space in support of a QbD filing with regulatory agencies; and when optimizing conditions for established procedures. In the latter case there can be considerable financial impact by minimizing the charges of reagents and solvents and by optimizing conditions for small improvements in yield or product quality.[13] HTS compliments DoE and may be used to select reagents, as in screening various acids for an acid-mediated deprotection. DoE can measure the effects of ranges of variables, such as the effects of charging various equivalents of a specific acid over a range of temperatures.[14]

The results of DoE experiments are analyzed to find which factors influence the results in a statistically relevant manner and which of those are interdependent, if any. These analyses can be shown through half-normal plots (Figure 2), analysis of variance (ANOVA), and other means. Data from nonlinear studies of reactions can be shown in contour plots and response surface methodology (RSM, Figure 3). DoE software packages used for process optimization include *Design Expert* (Stat-Ease Inc.), *MODDE* (Umetrics), *DoE Fusion PRO* (S-Matrix Corp.), *STAVEX* (Aicos), Minitab (Minitab Inc.), and *JMP* (SAS). Most, if not all, of the sponsoring companies provide training, consulting services, and free online tutorials for their products. The software platforms can be used to set up DoE studies that range from the very simple to the very complex and from fractional factorial designs to RSM designs that include settings at multiple levels. The more advanced designs are typically used to preclude confounding interactions of variables, or when practical constraints render certain combinations of factors unfeasible to execute.[15]

Unlocking the full potential of a DoE is predicated on systematically proceeding through a prescribed work flow:

1. Define the Objective: What process issues are to be resolved? Frequently, the goal is to optimize a process, but sometimes the process conditions are "locked" and the goal is merely to understand the robustness around the existing conditions. In this case, robustness is defined as probing the impact (if any) of small changes in the continuous factors[16] on the outcome.

2. Define the Factors/Variables and Their Ranges: The determination as to which factors/variables to include in a DoE depends on resources. Clearly, more variables will engender more reactions, time, and materials. Thus, it is imperative to prioritize them, using existing process knowledge, into groups such as known to be impactful, suspected, possible, and unlikely. Then assign high and low settings for the factors selected for inclusion in the study, as is the case when using the common two-level factorial design. These settings need to be relevant, achievable, and practical. The end product of this exercise is creation of the *design space*.

3. Define the Responses: These are the measurable outcomes of the process. Frequently, reaction yield (either isolated or assay) and conversion are the primary outputs. The responses are related to the objectives, and several can be included in a single DoE study *without* creating more reactions. Accuracy and reproducibility of experimental technique and assays are of paramount importance in order to achieve meaningful results. Typically, the center point experiments[17] are performed in triplicate as a probe of reproducibility but also serve to detect nonlinearity in a response when used in a *screening design* (vide infra).

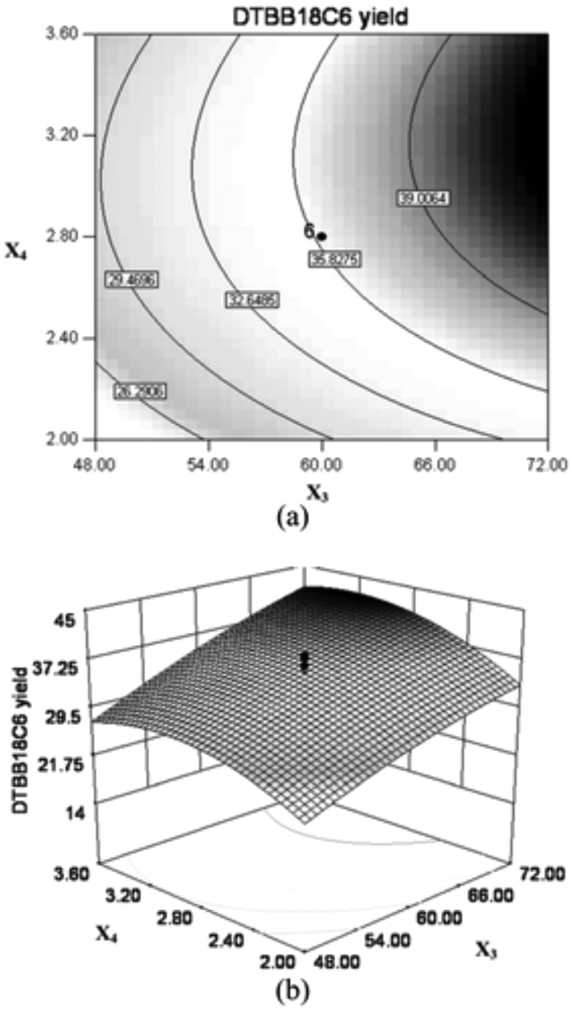


Figure 3. Contour (a) and three-dimensional response surface (b) plots of the effect of time (X_3) and equivalents of base (X_4) on reaction yield. Reprinted with permission from Fan, J.; Yi, C.; Lan, X.; Yang, B. *Org. Process Res. Dev.* **2013**, *17*, 368. Copyright 2013 American Chemical Society.

4. Select the Experimental Design: The choice depends on the objective, number of factors, and resources available, mostly related to the number of reactions required. The most common are screening designs in which *qualitative* information about the relevant factors is obtained. These designs also enable the factors to be ranked in order of extent of impact on the response. Screening designs are frequently employed to weed out the irrelevant factors to enable focusing on the most relevant factors in a second DoE design, also known as optimization designs.[18] These latter designs require more experiments than screening designs but generate a more comprehensive model of the response surface.

5. Generate Reaction Worksheet: Inputting all the above information into the software will generate a list of reactions to perform. Ideally, the reactions are all performed at the same time, but often this is impractical; thus, it is best have the software randomize the run order as a means of mitigating any systemic bias or error.

6. Perform Reactions/Collect Data: Again, it is critical to perform the reactions under identical experimental and analytical conditions. Sometimes it is not possible to do so; for example, perhaps two lots of starting material must be used out of necessity, as one has been depleted. DoE is able to account for this by dividing the reactions into "blocks" with each lot of starting material comprising a block. The statistical output is then able to determine whether the source of starting material imparted any bias into the design.

7. Data Input/Software Analysis: The results are input into the software, and each response is analyzed individually. A mathematical model is selected by the user based upon key metrics, such as the adjusted and predictive R-squared terms. The model can then be used to predict the outcome of a theoretical set of reaction conditions within the design space. Often there is more than one response requirement needed to meet a goal or specification. For example, a conversion must be >98.0 A% while the level of an impurity must be <1.5 A%. The software can assist in identifying reaction conditions that meet both requirements. These overlapping outputs are commonly known as "sweet spot plots".

8. Confirming Reaction: It is important to note the ideal conditions suggested by the DoE analysis are only a model prediction and must be verified experimentally to validate the results.

This review summarizes selected process research examples using DoE published in *Organic Process Research & Development* between 2003 and 2013.[19] The review aims to demonstrate the power of DoE to optimize processes with relatively few experiments, and it highlights the optimal process conditions that may not have been obvious at the beginning of research efforts. The discussions below are loosely grouped by reaction type or operation, such as hydrolysis, crystallization, and analytical development.[20] Some perspective is provided, as sometimes process scientists in these examples have used other processing considerations to select optimal scale-up conditions that were not evident based on the results of DoE studies. This review will not discuss the details of the statistical analysis.

For each summary, we sought to provide the following information if it was presented:

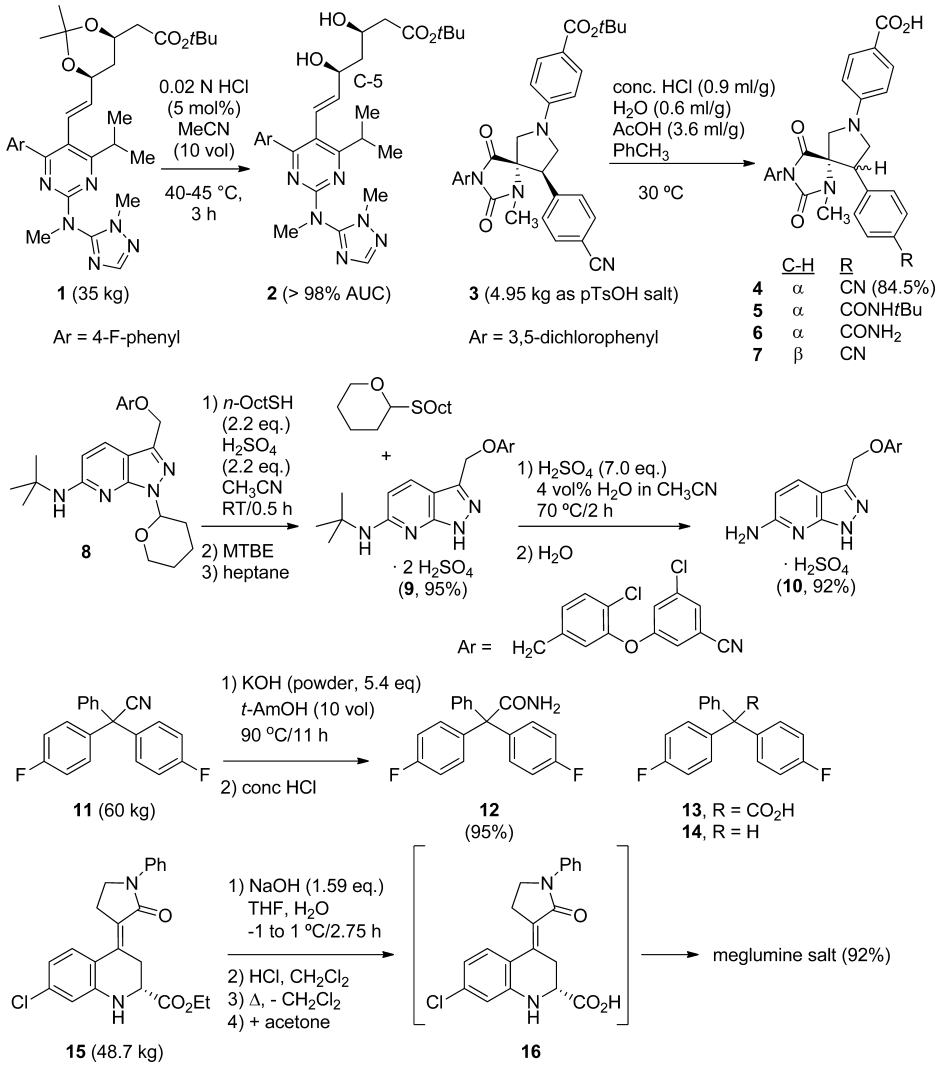
- What was the problem that was solved/studied?
- What type of DoE design was employed (e.g., screening, RSM, robustness)?
- What continuous factors were investigated? What were the values/ranges?
- How many reactions were performed? In what time frame?
- What was the overall improvement realized through the DoE?
- What scale was the confirming reaction performed on?
- What were the optimal conditions used to verify the DoE analysis?[21]
- To what extent did the DoE model predict the actual outcome?

REVIEW OF SELECTED DOE MANUSCRIPTS PUBLISHED IN OPRD FROM 2003−2013

Hydrolysis. Bristol-Myers Squibb (BMS) workers optimized the deprotection of acetonide **1** to diol **2** in the preparation of BMS-644950, an HMGR inhibitor (Scheme 1).[22] The parameters examined were the charge of MeCN (7.5−12.5 volumes), HCl concentration (0.015−0.030 N), HCl stoichiometry (0.03−0.07 equiv), and reaction temperature (35−49 °C). The data analysis revealed that temperature had the greatest impact on the reaction rate, with HCl concentration also having an impact. A competing epimerization at the C-5 hydroxyl was minimized using a catalytic amount of HCl at moderate

dx.doi.org/10.1021/op500169m | *Org. Process Res. Dev.* 2015, 19, 1605−1633

Scheme 1. Hydrolysis



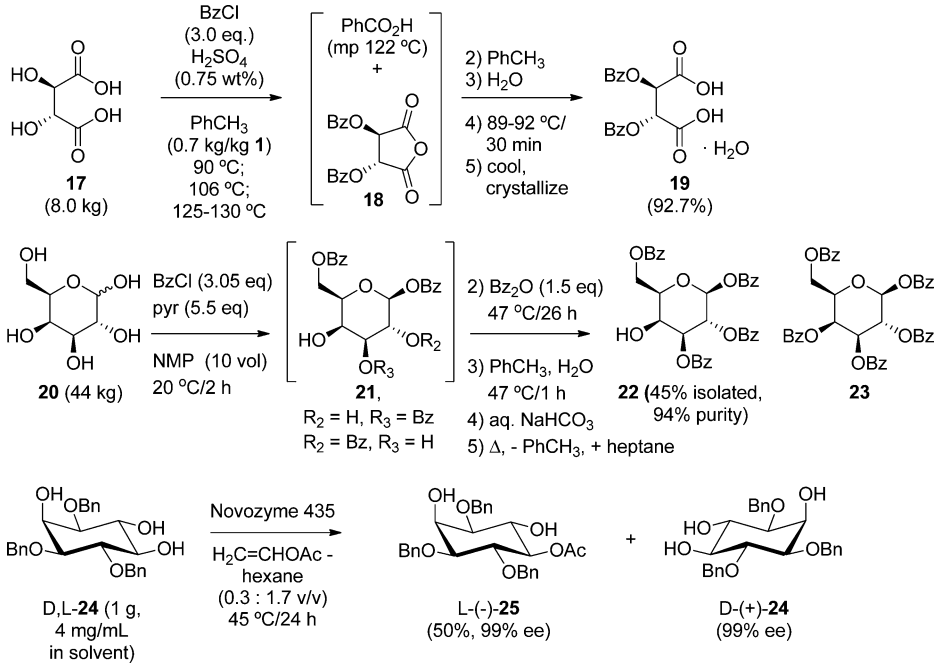
temperature. The diol ester was telescoped and converted to the ammonium carboxylate as the API.

Hydrolysis of the *tert*-butyl ester **3** led to the formation of 1% of the *tert*-butylamide impurity **5**, along with impurities **6** and **7** (Scheme 1), under the conditions initially used for scale-up (concentrated HCl and AcOH, *ca.* 1:2, in toluene at 55−65 °C).[23] The *tert*-butylamide was probably formed by a Ritter reaction of nitriles **3** or **4** with the cation generated through deprotection of the *tert*-butyl ester. Results from the initial DoE screen showed that the interaction of higher temperatures and higher concentrations of HCl had the biggest impact in increasing the rate of deprotection. The BMS group undertook additional DoE experiments to examine the effects of HCl and H_2O on the formation of **5**, **6**, and **7** (central composite design: HCl: 1.23, 1.57 mL/g of **5**; H_2O: 0.325, 1.175 mL/g of **5**). By increasing the concentration of water and decreasing the concentration of HCl, impurities **5**, **6**, and **7** were controlled to less than 0.25% in the API **4**.

DoE screening was used to investigate the removal of the *N*-THP and *N*-*t*-Bu protecting groups in the development of the reverse transcriptase inhibitor candidate **10** (Scheme 1).[14] Initial conditions provided the tosylate salt analog of **10** in about 65% yield through double deprotection of **8** using 5 equiv of dry TsOH and 25 equiv of TFA in acetonitrile. The use of either acid alone led only to loss of the THP group, and acetonitrile was necessary for double deprotection with minimal levels of impurities. The primary disadvantages of the initial conditions were the need to dry the reaction mixture azeotropically, the need for a large excess of TFA, and the relatively low yield. Through two high-throughput screenings for acids using microtiter plates, conc H_2SO_4 was identified as a simpler alternative for double deprotection; however, under those conditions, the yield of **10** was still about 65% with a large amount of black polymeric material observed, as with the initial conditions. Treating **8** with conc H_2SO_4 at RT smoothly removed only the THP group and produced black polymeric material; when purified **9** was treated with conc H_2SO_4 in acetonitrile at 70 °C, the yield of **10** was essentially quantitative. Thus, the low yields from double deprotection were due to the removal of the THP group, not the *tert*-butyl group, as had been suspected. The Merck researchers screened reagents to trap the byproducts from the THP group, i.e., dihydropyran and hydrated dihydropyran. When *n*-octanethiol was added to solutions of **8** in acetonitrile containing conc H_2SO_4, **9** was rapidly generated and crystallized in high yield; however, heating a mixture of **8**, *n*-octanethiol, and conc H_2SO_4 in acetonitrile produced lower yields of **10**, probably because the dihydropyran−thiol adducts were unstable. A DoE study to optimize removing the *t*-Bu group

dx.doi.org/10.1021/op500169m | *Org. Process Res. Dev.* 2015, 19, 1605−1633

Scheme 2. Acylations by Oxygen Nucleophiles



from **9** with a full-factorial design was executed varying water content (0, 10 vol %), temperature (55, 75 °C), reaction time (2, 8 h), and charge of H_2SO_4 (3, 15 equiv); three center point runs were added for a total of 19 runs. The highest yields were observed at the center point runs. A second DoE (optimization) was carried out, holding the temperature constant at 65 °C with a reaction time of 3 h; the design was a face-centered central composite (CCF) for water content (2, 4.5, and 7 vol %) and H_2SO_4 charges (5, 7, and 9 equiv), totaling 11 runs with three center point experiments. Under all conditions, amine **9** reacted completely, and the charge of H_2SO_4 had little effect on conversion or yield. Smaller charges of water raised the yields somewhat. Although the highest yield was found using 2 vol % H_2O, experiments showed that the yields dropped off sharply at charges of less than 2 vol % H_2O, and for robust operations on scale, a charge of 4 vol % H_2O was selected. Additional investigations showed no hydrolysis of the nitrile group in **10**. AcOH and *tert*-butyl acetamide were found in the reactions, indicating that acetonitrile was reacting with water and the *t*-$BuNH_2$. Overall the yield from the two-step processing was improved from about 65% to ~85%.

A collaboration between AMRI Global and Icagen optimized the final step in the synthesis of potassium ion channel blocker ICA-17043 (**12**) using DoE (Scheme 1).[24] The initial hydration of penultimate **11** was accomplished under acidic conditions but was plagued by formation of **13** and **14**, as well as a troublesome workup. Under basic conditions (powdered KOH in *tert*-amyl alcohol), their preliminary results showed a 95% conversion with 4% impurities. A 2^2 full factorial design (run in replicate with two center points; 10 reactions) was established to look at maximizing yield and minimizing impurity formation by varying solvent volume (4 and 7 vol) and KOH charge (1.5 and 3.5 equiv). The reactions, performed in a 12-position carousel reactor, were assayed after 6 and 24 h at 100 °C. Each factor and the two factor interaction impacted the reaction conversion, which was maximized with high KOH charge and low solvent charge. Impurity formation was reduced under the higher dilution conditions, while prolonged heating decomposed the product, especially with more KOH and less solvent. The conversion ranged from 76−100% over the design space. Additional post-DoE optimization led to the eventual optimal conditions that balanced the need for high conversion (the nitrile was difficult to remove) and low impurity formation.

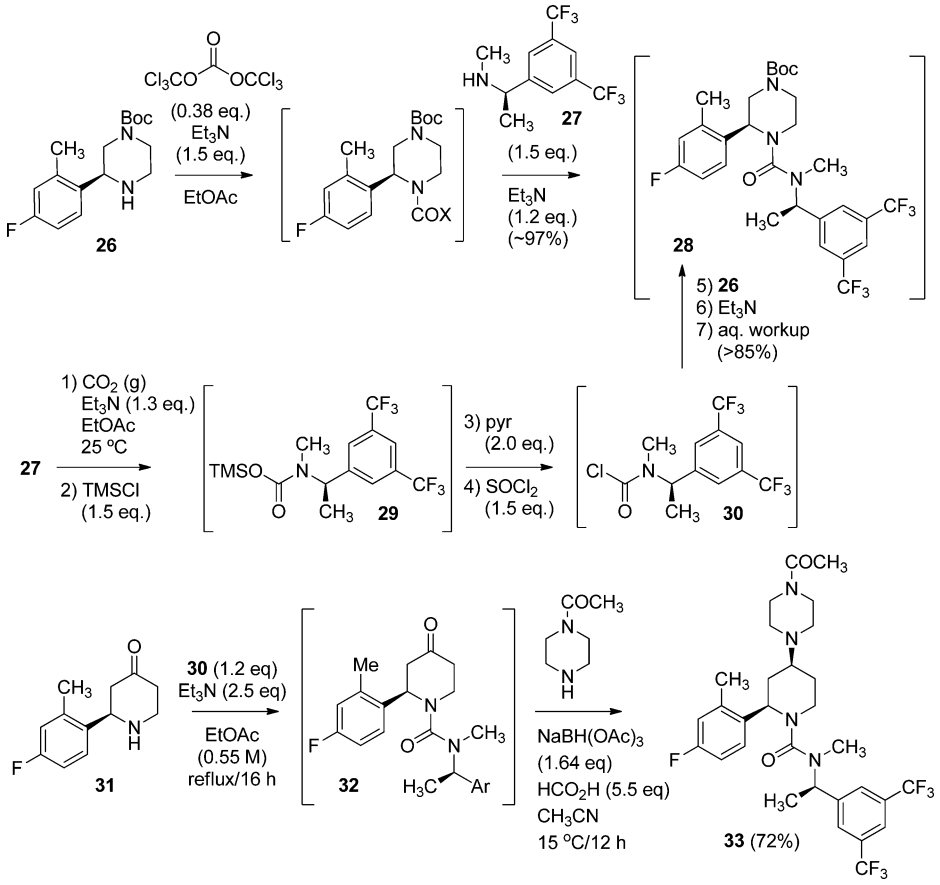
The hydrolysis of chiral ester **15** was investigated using DoE, in order to minimize racemization of **16** (Scheme 1).[25] Of the four factors studied (temperature, solvent amount, NaOH equiv, and addition rate), a low reaction temperature (0 °C) was the most important; only 0.3% epimerization was detected.

Acylation. For a large-scale preparation of *O*,*O*′-dibenzoyl-L-(+)-tartaric acid (**19**; Scheme 2), DoE studies were used to optimize the benzoylation of **17** and the subsequent hydrolysis of intermediate anhydride **18**, increasing the yield from 81% to 95% in the telescoped process.[26] Twenty runs were carried out using a central composite design with 6 center points looking at solvent amount (0.65−1.15 g of toluene/g of **17**), acid equivalents (15−35 mg of H_2SO_4/g of **17**) and temperature (105−125 °C). The results were correlated with the isolated yield of and the amount of color in **19** (measured by absorption at 600 nm). The yield increased as the temperature was raised and the charges of toluene and H_2SO_4 were decreased, while the color increased as the charges of toluene and H_2SO_4 were increased. To prepare **18**, the reaction temperature was raised in stages, first to 90 °C to accelerate the reaction, then to 106 °C to encourage the release of gaseous HCl, and finally to 125−130 °C to melt the benzoic acid byproduct. Next the reaction mass was cooled and diluted with toluene and water, and the mixture was heated at reflux at 89−92 °C to hydrolyze anhydride **18**. [Safety note: massive evolution of vapors would occur if water is added to the mixture above the boiling point of the toluene-water azeotrope.] The mixture was cooled to crystallize **19**. This process was carried out in a 1.5 m^3 reactor.

A Sanofi-Aventis group[27] studied the conditions to optimize the selectivity and conversion for the tetrabenzoylation of D-galactose (**20**) to produce **22** as part of their synthesis of SGLT inhibitor candidate SAR7226 (Scheme 2). An initial solvent screen identified NMP and DMA as the most desirable solvents

dx.doi.org/10.1021/op500169m | *Org. Process Res. Dev.* 2015, 19, 1605−1633

Scheme 3. Acylations with a Carbamoyl Chloride



to minimize formation of both the over-reacted pentabenzoyl species **23** (10−13 A%) and the tribenzoylated species **21** (5−8 A%). NMP was chosen for further development for industrial hygiene reasons. Benzoyl chloride was found to be more reactive and less specific than benzoic anhydride. The goals became to benzoylate with BzCl at the more reactive hydroxyl groups (C1 and C6) and better solubilize the intermediates, and then complete the benzoylation to **22** through the more selective Bz_2O. Thus, the two benzoylating reagents were employed in tandem in the DoE study, BzCl and Bz_2O (1.5 equiv), while pyridine (5.5 equiv) was selected as the base.

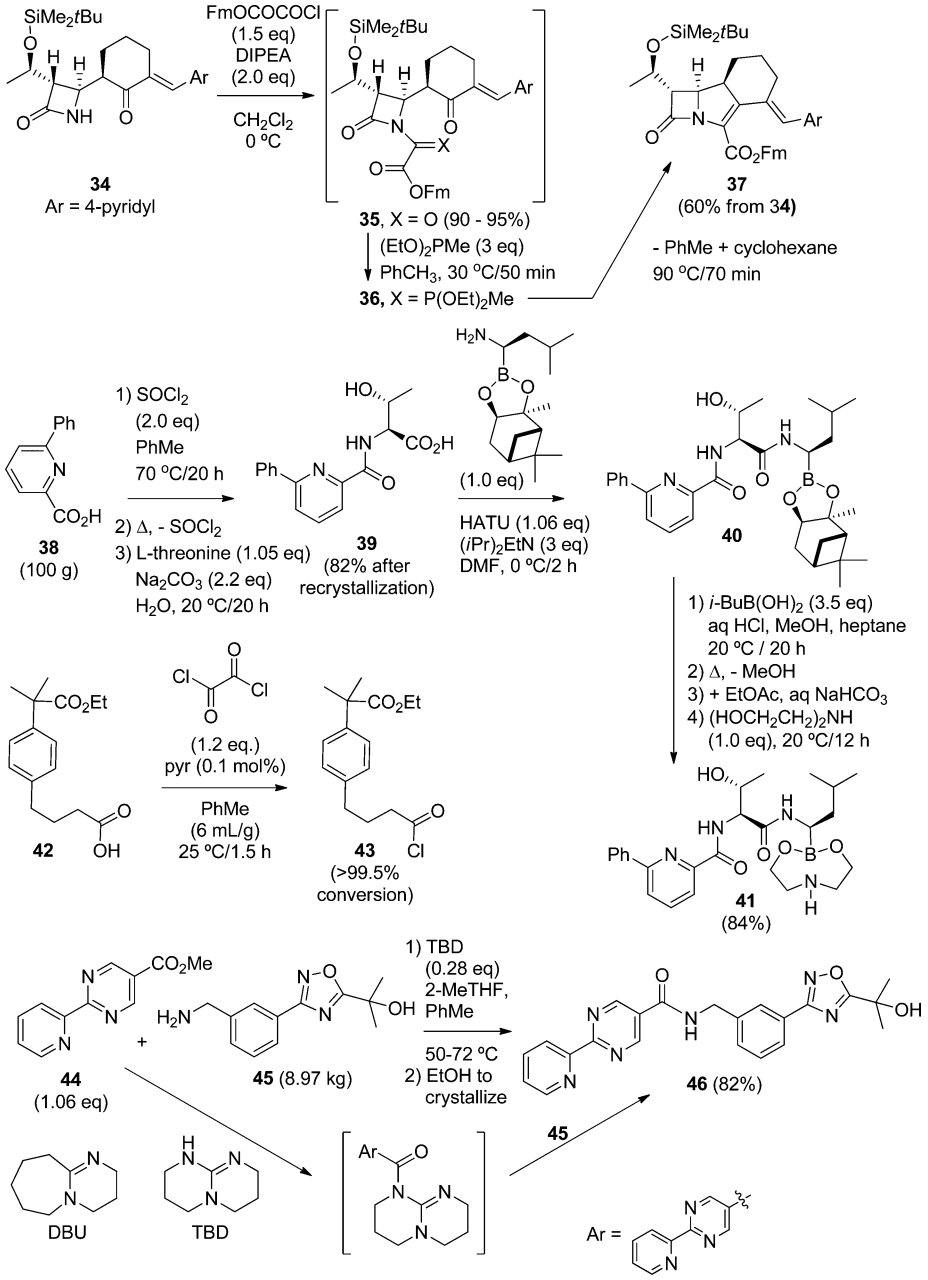
The authors chose an optimization design (second-order central composite) looking at the BzCl stoichiometry (2.9−3.2 equiv), solvent volume (7−13 volumes), and reaction time (14−24 h) at 20 °C. The target was keeping the level of **23** to <15 A% while maximizing conversion, and then adding the final benzoyl group with Bz_2O at a moderate temperature. The data indicated that the highest levels of the desired product (the thermodynamically equilibrated β-anomer **22**) were obtained with 10 volumes of NMP and 3.05 equiv of BzCl and Bz_2O (1.5 equiv). The optimized process provided a mixture of **22** (65−70 A%), **23** (8−11 A%), and **21** (5−9 A%). The DoE study served to identify robust conditions that reduced the formation of the over-reacted species from 13 A% to 8−11 A%.

A group from universities in Brazil optimized the kinetic resolution of 1,3,6-tri-*O*-benzyl-*myo*-inositol (D,L-**24**) by acetylation using Novozyme 435, a lipase immobilized on acrylate resin (Scheme 2).[28] In the first DoE screen, a fractional factorial design ($2^{4-1}$with three center point runs; 11 reactions) was employed with the goal of maximizing the conversion; factors varied were substrate concentration (2−14 mg/mL), vinyl acetate concentration (187−560 mg/mL), enzyme concentration (54.6−136 units), and temperature (30−50 °C). The concentrations of the enzyme and substrate had the greatest impact on the conversion, while temperature and vinyl acetate charge (a large molar excess) had virtually no impact. The conversion in the design space ranged from 6−43%. In a subsequent central composite optimization design, the acyl donor charge was fixed; the conversion was greatly influenced by the temperature and substrate concentration. An interdependence between substrate and enzyme concentrations was observed. Reaction conditions were selected for high conversions with relatively low enzyme loading. The model predicted highest conversions at 57 °C, but that temperature was not used due to concerns about increased enzyme degradation. A portion of enzyme was reused seven times under optimal conditions before the extent of conversion fell off significantly. Overall, these optimizations reduced the time cycle from 112 to 24 h and increased the productivity 15-fold.

GSK researchers used DoE to optimize the formation of urea **28** (Scheme 3).[29] Triphosgene was the only reagent found to react with hindered amine **26** to form an active carbonyl derivative and then generate **28** from amine **27** in an acceptable yield. DoE studies were carried out, varying the triphosgene charge (0.35, 0.45 equiv), solution volume of **26** (2, 10 vol), benzylamine **27** charge (1.2, 1.5 equiv), and solution volume of **27** (5, 15 vol); using a central composite design, 30 experiments were run. To minimize the generation of impurities, the optimal conditions required 0.38 equiv of triphosgene and 1.4 equiv of benzylamine **27**; solvent volumes were not critical. To avoid the

dx.doi.org/10.1021/op500169m | *Org. Process Res. Dev.* 2015, 19, 1605−1633

Scheme 4. Acylations by Nitrogen Nucleophiles

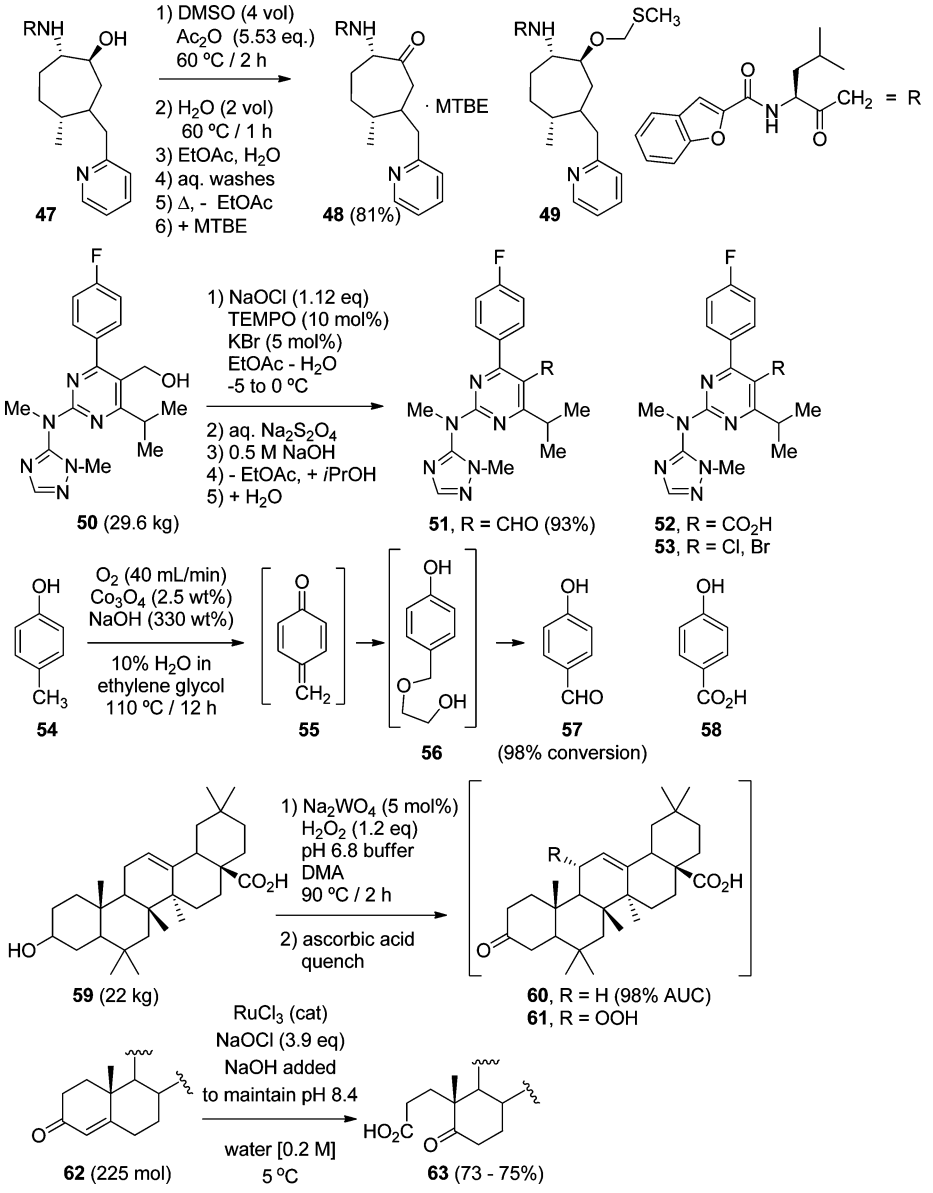


use of phosgene or phosgene derivatives, a second-generation approach was developed. Reaction of amine **27** with CO_2, Et_3N, and TMSCl probably generated the soluble TMS-carbamate **29**, which was then treated with pyridine and $SOCl_2$ to generate the carbamoyl chloride **30**. The latter was then condensed with amine **26** to generate urea **28**. A DoE examined the effects of varying seven factors in this reaction mixture of gas, liquid, and solid salts: temperature before and after TMSCl addition, volume of EtOAc, and the charges of Et_3N, TMSCl, pyridine, and $SOCl_2$. Twenty experiments were run in a D-optimal design, keeping the flow of CO_2 constant throughout the processing. Higher charges of $SOCl_2$ increased the yields but produced darker reaction mixtures. Concentrated reactions and lower temperatures gave lower yields. The CO_2–$SOCl_2$ process was used to manufacture the API through deprotection of **28** and salt formation.

GSK workers applied QbD principles in developing the manufacturing process for casopitant mesylate, an NK_1 antagonist.[30] They sought to determine the proven acceptable ranges (PAR) for the parameters known to impact the quality of the API using DoE for two of the eight steps. In one stage (Scheme 3), (*R*)-piperidone **31** was acylated with carbamoyl chloride **30** to yield urea **32**. Four process variables were studied: reaction concentration in EtOAc (4.5–5.5 vols), TEA equivalents (2.3–2.7), carbamoyl chloride equivalents (1.0–1.2), and reaction temperature (75–85 °C). The yield response varied over these reaction ranges from 82 to 99%. Analysis indicated that only the amount of reagent **30** had an impact on yield, with 1.2 equiv being preferred. The subsequent reductive amination of urea **32** with 1-acetylpiperazine to generate **33** was also optimized with DoE to determine the impact on the downstream API. A two-level fractional factorial design (2^{6-3}) covering six variables with two center points was undertaken (10 reactions); the responses measured were yield and diastereomeric ratio. The six variables were $NaBH(OAc)_3$ equivalents

dx.doi.org/10.1021/op500169m | *Org. Process Res. Dev.* 2015, 19, 1605–1633

Scheme 5. Oxidations



(1.55−1.72), formic acid equivalents (5−6), reaction temperature (5−25 °C), age period for the $NaBH(OAc)_3$ and formic acid (5−55 min), 1-acetylpiperazine equivalents (2.0−2.5), and addition time (5−55 min). The center point values for the $NaBH(OAc)_3$, formic acid, and piperazine equivalents at 15 °C gave the highest yield (ca. 72%) and were selected for the manufacturing process. Minimal variation of the diastereoselectivity was observed within this design space (maximum: 75:25). The optimized procedures are described for a 1 kg scale in the paper.

GSK workers optimized an acylation and cyclization in the synthesis of GV143253A, a broad-spectrum antibiotic.[31] Initially the acylation of the β-lactam nitrogen of intermediate **34** with FmOCOCOCl proceeded in only 45−50% yield (Scheme 4). A central composite design (10 experiments with 2 center points) was set up to examine the effect of the reagent (1.3−2.2 equiv) and DIPEA (1.5−3.0 equiv). Parallel experimentation was facilitated by the use of an Anachem SK233 apparatus. The second reaction examined was the formation of phosphorane **36** from **35**, and another central composite design (20 experiments with 6 center points) was used. The three parameters were phosphonite equiv (2−10), solvent volumes (DCM/toluene; 10−50), and temperature (undisclosed range). Higher amounts of the phosphonite led to improved yields at 40 °C while the reaction concentration had no impact. The phosphorane was cyclized in cyclohexane at 90 °C to produce the desired crystalline intermediate **37** in 60% overall yield from **34**. This improved yield compares to the 45% isolated yield obtained before the second DoE optimization.

The Process Chemistry team from Teva employed DoE to optimize several continuous variables in the synthesis of **41** (Scheme 4), a derivative of delanzomid (CEP-18770), a peptide boronic acid under investigation as a proteasome inhibitor for the treatment of multiple myeloma.[32] Several DoEs using *Design Expert* were performed to optimize the operating range of each variable of the acylations to yield **39** and **40** and transesterification in the three step process. For the first step, the thionyl chloride charge was reduced from 5 to 2 equiv under more concentrated conditions, and by charging more than 1.0 equiv of L-threonine and 1.3 equiv of sodium carbonate, the

dx.doi.org/10.1021/op500169m | *Org. Process Res. Dev.* 2015, 19, 1605−1633

formation of a troublesome impurity was avoided. To minimize epimerization at the threoninamide methine of **39** in the second acylation, HATU was employed and at least 2.8 equiv of DIPEA was necessary. The other factors were found to be robust. For the transesterification, the amount of isobutylboronic acid (3.5 equiv) and concentrated conditions were imperative to driving the transesterification equilibrium in a timely manner. The collective process improvements resulted in an improved overall yield (25 to 60%) and product quality, and avoided chromatography.

Lilly researchers used a full factorial DoE to optimize the acid chloride formation of **42** (Scheme 4).[33] Pyridine was selected as the catalyst over DMF, as the latter produced an impurity that was difficult to purge. The three factors examined were temperature (20, 40 °C) and the charges of oxalyl chloride (1.05, 1.3 equiv) and toluene (4, 9 volumes). A higher charge of oxalyl chloride and a higher temperature promoted the formation of the acid chloride **43**, while, at lower temperature and a higher volume of toluene, the conversion to **43** was slower. Optimal conditions were selected near the center of the design space. Compound **43** was subsequently used to acylate a hydrazine derivative.

Using 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) as an acylation catalyst, a Sanofi team optimized the formation of amide **46** from ester **44** and benzylamine **45** (Scheme 4). DBU or tetramethylguanidine gave <8% conversion in reaction screening.[34] A three factor central composite design studying the effects of TBD catalyst (0.10−0.33 equiv), reaction temperature (60−80 °C), and reaction time (3−5 h) was undertaken to maximize conversion while minimizing the catalyst charge. The analysis revealed the TBD charge was the most influential factor, followed by temperature. The best combination of robustness and the above criteria was satisfied with 0.28 equiv of TBD at 70 °C for 4 h, using a stepwise heating protocol. These simple conditions skirted the need to prepare **46** in separate steps of hydrolysis and acylation.

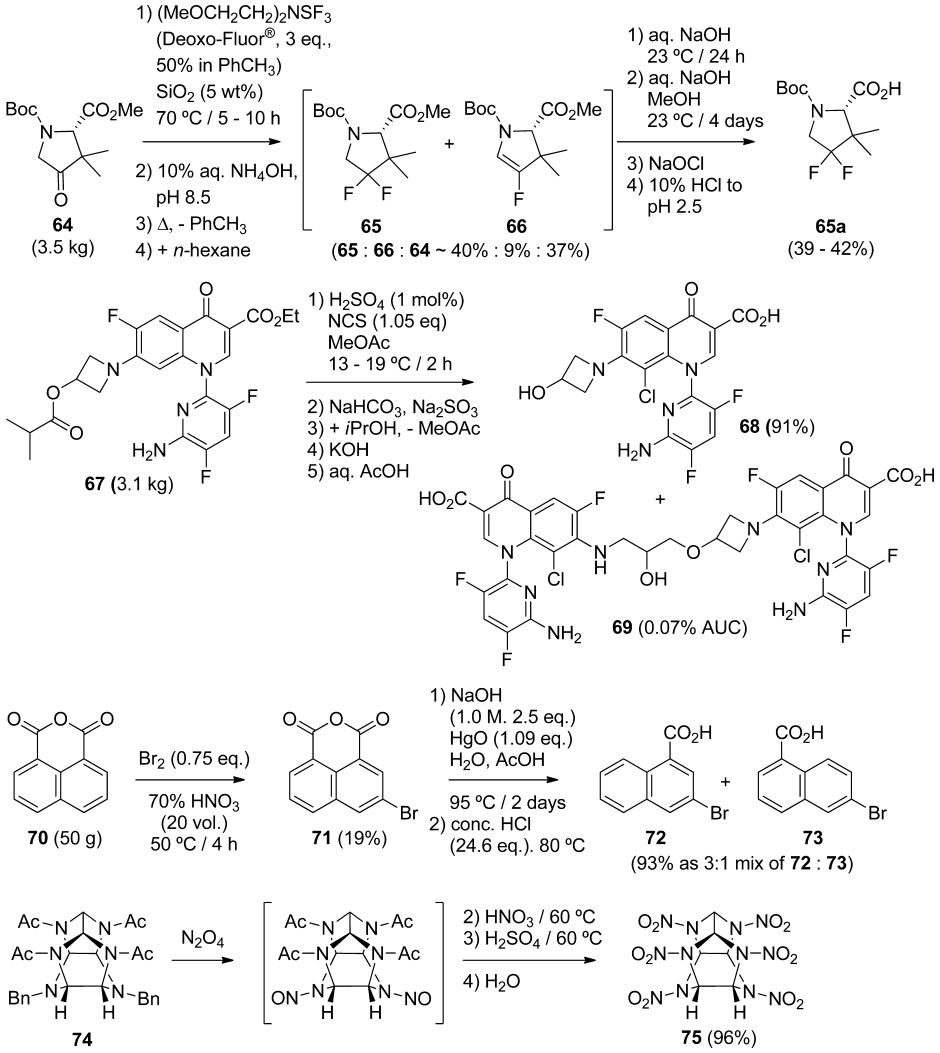
Oxidation. GSK researchers investigated in detail three procedures to oxidize the α-amino alcohol **47** to the ketone API **48** (Scheme 5).[35] Key considerations were conversions of ≥99.5% to ease purification, avoiding epimerizing the center α- to the ketone, minimizing environmental impact, and minimizing any impurities, especially metals. TEMPO oxidations were examined, varying the charges of TEMPO (0.05, 0.5 equiv), KBr (0.05, 0.5 equiv), and NaOCl (1.1, 2.0 equiv), along with the pH of the NaOCl solution (8.0, 10.0) and the agitation rate (300, 900 rpm) of the biphasic mixture (aqueous CH_2Cl_2). Under half-factorial experimental design, 32 experiments were carried out, while monitoring the disappearance of **47** and the formation of **48** and an impurity. The most critical factors for the optimal conditions included charging 0.35 equiv of TEMPO and acidifying the pH of commercial NaOCl solutions to about 9.0. Unfortunately, these conditions did not prove to be robust, and the NaOCl decomposed to unidentified byproducts, possibly O_2, Cl_2, or Br_2; hence, TEMPO-catalyzed oxidations were abandoned. Oxidation using a polymeric piperidinyloxyl radical (PIPO) catalyst derived from oxidation of Chimassorb 944 was also examined. Again 32 experiments were performed, varying the charges of PIPO (1.67, 6.67 wt %), KBr (0.03, 0.2 equiv), NaOCl (1.1, 1.8 equiv), pH (8.4, 9.8), and agitation rate (300, 900 rpm). High conversion with high selectivity was observed using 3 wt % PIPO, 0.2 equiv of KBr, and 1.1 equiv of NaOCl at pH 9.0. Unfortunately, the stable emulsion generated by the polymeric reagent posed a significant obstacle to further development, and the approach with PIPO was also abandoned. Under Moffatt oxidation conditions (Ac_2O−DMSO, shown in Scheme 5), the Pummerer side product **49** was the primary impurity, and its formation was promoted by increasing amounts of H_2O. A DoE was performed on four variables, equivalents of Ac_2O (2.0, 4.0), H_2O (0.0, 1.0), and DMSO (3.4, 4.5); and temperature (50, 70 °C); 32 runs were carried out, monitoring for conversion to **48** and formation of **49**. The charges of Ac_2O and H_2O were the most critical factors, with an optimum temperature of 60 °C. The authors mentioned that, before charging Ac_2O, a vacuum distillation with toluene can be used to azeotrope out water brought in with the DMSO or with **47**, thus decreasing the formation of byproduct **49**.

BMS workers found the TEMPO-promoted oxidation of alcohol **50** to aldehyde **51** was complicated by the formation of impurities **52** and **53**. An undisclosed DoE was performed leading to the conditions that identified the ideal reagent stoichiometries (TEMPO, 0.1 equiv; KBr, 0.05 equiv; NaOCl, 1.12 equiv). Unbuffered NaOCl was used to circumvent the decomposition of NaOCl seen with more acidic solutions often employed for TEMPO oxidations.[22]

DoE was used to examine the air-mediated oxidation of *p*-cresol (**54**) to *p*-hydroxybenzaldehyde (**57**) (Scheme 5).[36] Ethylene glycol was charged to trap the benzoquinone methide **55** as the glycol ether **56**, and this solvent was chosen, as it was safer than MeOH (based on flammability and flash point considerations), which was initially used. Water was beneficial to the reactions, perhaps because it decreased the viscosity of the solvent. Four factors were examined under DoE screening: the charges of substrate (15, 25 g), base to substrate ratio (2:1, 4:1), water charge (10%, 20%), and temperature (80, 90 °C). A reduced central composite design was carried out, with a total of 21 experiments, monitoring for the formation of **56** and **57**. An increased substrate loading decreased the selectivity for **57**. Analysis of the DoE results indicated that the process optimum would fall outside the initial ranges chosen, so further optimization was undertaken. Under optimal conditions, **57** was generated in 98% yield, and no *p*-hydroxybenzoic acid (**58**) was detected.

The oxidation of alcohol **59** to ketone **60** was optimized by Shionogi workers[37] en route to the synthesis of endothelin A receptor antagonist S-0139 (Scheme 5). The Jones oxidation initially developed was deemed unsuitable for large-scale production, so it was replaced with a more benign sodium tungstate−H_2O_2 reagent.[38] The reaction with Na_2WO_4−H_2O_2 initially proceeded in 81% yield in dimethylacetamide (DMA) but was plagued by the formation of the hydroperoxide **61**, which led to lactone, diketone, and epoxide side products. A central composite design (16 reactions) was chosen to study the effect of hydrogen peroxide (35% aqueous; 1.0−1.4 equiv) and sodium tungstate (3−7 mol %) stoichiometry as well as the pH of the phosphate buffer employed (1.5−9.1) in the reaction. The reactions were performed in 0.4 M aqueous DMA at 90 °C on a 2.4 mmol scale. The responses were yield of the desired ketone **60** and formation of the difficult-to-reject peroxide **61**. The results showed that the impurity formation was minimized at high pH and with fewer equivalents of hydrogen peroxide. The yield of the ketone was maximized at 1.2 equiv of oxidant over a wide pH range using 5 mol % of the tungstate. Due to the instability of the reagent system at higher pH, a buffer of pH 6.8 was selected for the manufacturing conditions; the reaction mixture was quenched and ozonolyzed to the desired product (not shown).

Scheme 6. Halogenation and Nitration

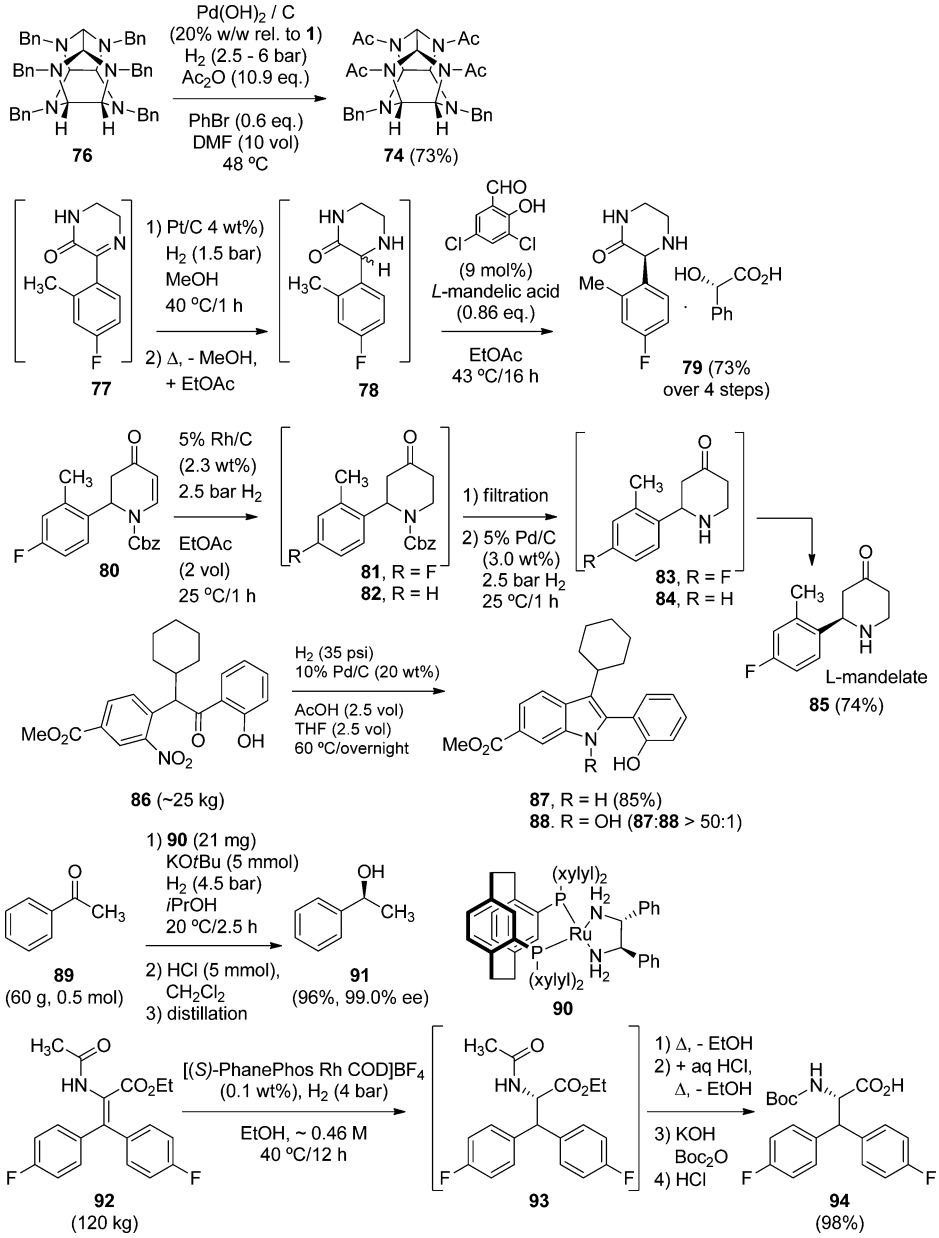


The oxidation of keto-olefin **62** to keto-acid **63** was optimized by a Lonza group en route to an undisclosed azasteroid (Scheme 5). A CCF design was employed, with 16 reactions including 2 center points.[39] The authors noted that unless the poorly soluble starting material was micronized, the reaction was very sensitive to agitation and amount of $RuCl_3$ catalyst. The continuous variables were solvent volume (300−600 mL normalized to a 100 mmol charge of **62**), NaOCl equiv (3.6−4.4), and reaction pH (7.7−8.9), with an unspecified charge of $RuCl_3$ catalyst. The exothermic nature of the reaction dictated the temperature be fixed at 5 °C. The responses modeled were yield, formation of three unidentified overoxidized side-products, and conversion (unreacted **62**). The level of the side-products needed to be kept <0.1%. The data suggested that reaction concentration was not critical if the other two factors (NaOCl equiv and pH) were within their optimal range. A higher charge of NaOCl led to higher conversion with higher impurity formation. Since the starting material was effectively removed during the crystallization, the response of conversion of starting material was removed from the analysis, which predicted the optimal values to be 510 mL of solvent, 3.9 equiv of NaOCl, and pH 8.4. The expected yield of 78% was verified on a 1.5 mol scale and gave a 74% yield of **63**. Subsequent production scale (12 batches, 225 mol each) gave yields of 73−75%, with the levels of the side-products and **62** at <0.03%.

Halogenation and Nitration. A Pfizer group required a robust process for the synthesis of *gem*-difluoro compound **65** en route to an HIV protease inhibitor candidate (Scheme 6).[40] The fluorination of ketone **64** produced the vinyl fluoride **66** in addition to desired **65**, and various treatments were attempted to convert **66** to **65**. The following reagent stoichiometry ranges were studied: fluorination with Deoxo-Fluor (1.6−3.0 equiv), DCE (0−0.68 mL/mmol of **64**), water (0.1−0.3 equiv), Aliquat 336 (0.1 equiv), $NaHCO_3$ (0.2−0.6 equiv), and either spray-dried or amorphous KF (1−2 equiv). All the reactions were run in toluene (1.3 M) at 70 °C. Other reagents and additives studied included $AlCl_3$, 18-crown-6, and TBAF, all of which led to partial or complete degradation of **64**. A fractional factorial design with 2 center points was utilized (18 reactions) with an HEL Duet 317 automated parallel reactor setup. The only factor that impacted the yield was the Deoxo-Fluor charge, with the higher amount (3 equiv) leading to better yields and helping to decompose **66**, thus easing workup. Subsequent investigations showed that $NaHCO_3$ was required to buffer the reaction acidity; under optimized conditions, ∼37% of starting material remained. Subsequently, the researchers found that a small charge of silica gel promoted fluorination in nonglass reactors, probably through the

dx.doi.org/10.1021/op500169m | *Org. Process Res. Dev.* 2015, 19, 1605−1633

Scheme 7. Hydrogenations



generation of a fluorosilicate,[41] and the reaction and workup were optimized as shown in Scheme 6. Unreacted **64** was selectively hydrolyzed and purged by extraction; a second treatment with NaOH hydrolyzed **65** and **66**, leaving Deoxo-Fluor-related impurities in the organic phase. Treatment with NaOCl to decolorize solutions was found to decompose the carboxylic acid generated from **66.** Through these fortuitous findings, extractive workups were used, and chromatographic purification was avoided. Ten batches were carried out on scale through fluorinations in a Halar reactor.

In the chlorination of quinolone **67** catalyzed by sulfuric acid, the formation of the dimeric impurity **69** posed a concern (Scheme 6). In order to minimize its formation, DoE screening was used to examine the impact of reaction temperature (13, 21 °C), the equivalents of NCS (1.05, 1.2) and H_2SO_4 (0.8, 1.2 mol %), water content (0, 0.5 vol %), solvent (MeOAc or EtOAc), solvent charge (2, 3 vol), and NCS addition rate (0.05, 0.3 vol/min). Only 19 experiments were needed by using a 2^{7-3} fractional factorial design plus three center points. The formation of **69** was suppressed by charging higher levels of NCS, adding the reagent more quickly and by using dry solvents and lower temperatures. The hydrolysis of the azetidine intermediate was implicated by the impact of H_2O on the formation of **69**. Furthermore, by using MeOAc, a smaller excess of NCS was needed. DoE screening was then used to examine the robustness of the selected conditions to control the amount of **69**: reaction temperature (13, 21 °C), the equivalents of NCS (1.04, 1.07) and H_2SO_4 (0.8, 1.2 mol %), and NCS addition period (30, 75 min). Ten experiments were carried out (2^{4-1} and two center points). The first three factors significantly influenced the formation of **69**, but the conditions were judged robust enough to afford batches of **68** with suitably low levels of **69**. Upon scale-up in the kilo lab, the API (**68**) was generated in 91% yield as the N-Me-D-glucamine salt, with the level of the impurity **69** below the limit qualified in a tox batch.[42]

dx.doi.org/10.1021/op500169m | Org. Process Res. Dev. 2015, 19, 1605−1633

Researchers at AstraZeneca used DoE studies to examine conditions for the bromination of **70** and subsequent hydrolysis and decarboxylation of intermediate **71** (Scheme 6).[43] To optimize the yield of **71**, five factors were varied: Br_2 charge (0.75, 0.95 equiv), Br_2 addition time (10, 60 min), 70% HNO_3 charge (15, 20 vol), reaction temperature (50, 65 °C), and reaction time (0.5, 4 h). A total of 11 experiments were run, 2^{5-3} plus three center points. Increasing the charge of Br_2 gave a higher yield but negatively affected the quality. For safety reasons, the solvent volume was not decreased below 20 volumes. Although the DoE investigations did not improve the yield of **71** from the initial conditions, the process proved sufficiently robust to provide material needed for further studies. DoE studies were then used to investigate the conversion of **71** to acid **72**. Six factors were investigated: the charges of NaOH (2.5, 4.0 equiv), H_2O (6.75, 13.25 vol), HgO (1.08, 1.15 equiv), AcOH (1.0, 2.0 vol), HCl (10, 20 vol), and a second charge of H_2O (0, 12.5 vol). No conditions were developed that improved the ratio of **72**:**73** above 3:1, and **72** was purified by recrystallization from AcOH.

The preparation of the energetic molecule HNIW (CL-20, **75**)[44] was optimized using orthogonal array designs, a type of fractional factorial design (Scheme 6).[45] Debenzylation/nitrosation of **74** was carried out by treatment with N_2O_4, and after the sequential additions of HNO_3 and H_2SO_4, the hexa(nitroamine) **75** was generated. Nine parameters were examined (equivalents of N_2O_4, HNO_3, and H_2SO_4; temperature; time for nitrosation; acid addition temperatures; and addition times for the acids), varying four levels of each parameter. A full factorial design would have totaled 262,144 experiments (4^9), but using the powerful orthogonal array design, a total of only 32 experiments were carried out. The analysis showed that only the time (10 h) and temperature of nitrosation, and the sulfuric acid addition temperature impacted the yield. A 96% yield of **75** was obtained under the optimal conditions.

Reduction. The debenzylation and in situ N-acetylation of hexaazaisowurtzitane derivative **76** to yield **74** was optimized using a reduced central composite design (CCD) (Scheme 7).[46] The factors studied were the effects of Pd catalyst loading (8–17%), reaction temperature (39–51 °C), hydrogen pressure (3.2–5.3 bar), and Ac_2O stoichiometry (9.1–13.6 equiv) on reaction yield. The 21 experiments were performed on a 5 g scale in DMF (10 vol) and yields ranged from 8–70% over the design space. Of the four factors studied, only the hydrogen pressure did not have an influence on the yield. Higher catalyst loading led to better yields, as did temperatures around 48 °C and an acetic anhydride charge of 10.9 equiv. Bromobenzene was charged as a precursor to HBr, which catalyzed the debenzylation. The model predicted a 75% maximum yield, which was validated by a confirmatory experiment that gave **74** in 73% yield.

A GSK team in Italy used DoE for both the reduction of imine **77** and the subsequent resolution of **78** to optimize the route to ketopiperazine **79**, an intermediate in the synthesis of GW597599, an NK-1 antagonist (Scheme 7).[47] Initially the hydrogenation of **77** with Pd in MeOH required prolonged reaction times, high catalyst loading, and high pressure. A catalyst change to Pt/C, followed by a DoE of the continuous variables (hydrogen pressure: 0.3–4.3 bar; temperature: 20–40 °C; catalyst charge: 2–10 wt %) enabled an improved procedure (Scheme 7) with complete reduction within 1 h. The reduction was telescoped with a crystallization-induced dynamic resolution (CIDR)[48] and provided a 70% yield of the desired enantiomer as the mandelate salt on a 5 L lab scale; upon scale-up to the pilot plant, the yield dropped to 50%, in part because the batch adhered to the surface of the crystallizer. Thus, a full factorial design studying the effects of agitation rate (200–1000 rpm), water charge (0.10–0.45 equiv), and aldehyde charge (0.03–0.10 equiv) was undertaken. The agitation most affected yield, implicating the importance of thorough mixing. Computer modeling suggested that poor agitation behind the baffles was responsible for the encrustation found on scale with the CIDR, and for that thick suspension a conical vessel without baffles was selected to avoid dead-mixing zones. The process was successfully scaled up in a pilot plant.

GSK workers needed to understand the factors that led to the formation of the des-fluoro impurity in casopitant mesylate.[49] This impurity was detected at 0.1% in intermediate **85**, and controlling and minimizing its formation at no more than 0.15% were critical to meeting the API specification. The source was traced to the reduction of **80** (H_2 over Rh/C) that generated intermediate **81** and not the subsequent hydrogenolysis to **83** (H_2 over Pd/C) (Scheme 7). A full factorial design looked at the impact of 5% Rh/C loading (0.76–3.82%), hydrogen partial pressure (0.2–3.0 bar), EtOAc volumes (3–10) with two center points in a 10-reaction matrix. Reaction temperature (set at 25 °C) was not included, as prior experience showed it would not have an impact on the impurity formation. Data analysis indicated that the high settings for the catalyst loading and hydrogen pressure led to higher levels of **82**, while the solvent amount had no impact. These conditions, in concert with removing the Rh/C catalyst before the second hydrogenolysis, ensured control of impurity **84** and fulfilled requirements for control through QbD.

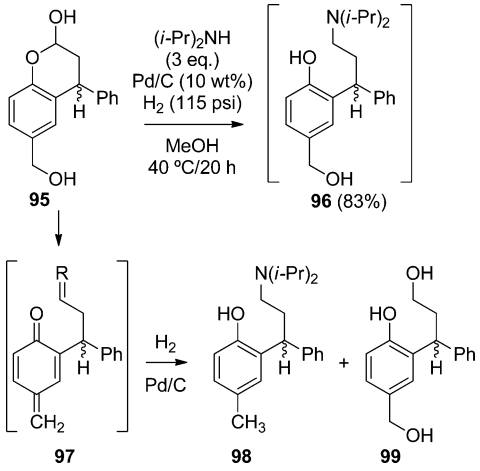
A Merck Process group in the U.K. used DoE to improve the reductive ring closure of intermediate **86** to prepare indole **87** while minimizing formation of **88**, the corresponding *N*-hydroxyl species (Scheme 7).[50] The formation of **88** was associated with stalled reactions, and the purity of **86** was also important for successful reactions. Initially, conversions were only about 50%, and so the researchers resorted to a fractional factorial design for rapid optimization. Using an Endeavor parallel hydrogenation apparatus, they studied the effects of temperature (40–75 °C), hydrogen pressure (10–70 psi), AcOH (0–50 vol %), and 10% Pd/C loading (10–20 wt %). High levels of AcOH and higher temperatures generally led to higher yields while pressures >50 psi correlated with lower yields. A model derived from the main effects proved useful in identifying reaction conditions for scale-up that allowed for decreased solvent charge and lower temperature.

Researchers from Chirotech investigated the asymmetric hydrogenation of acetophenone (**89**) using either of two enantiomeric precatalysts containing ruthenium and a diphosphine derived from a cyclophane to form **90**, for example.[51] The purpose of these experiments was to demonstrate that the hydrogenations could be carried out in high yield and with high chiral purity on a multigram scale to yield **91** (Scheme 7). A DoE screen was carried out, varying pressure (3, 13 bar), concentration of **89** (0.2, 0.4 g/mL), and temperature (10, 30 °C), while monitoring for stereoselectivity and rate; 11 experiments were carried out using a 2^3 design plus three center point runs. Under those conditions, there was slight variation in selectivity. Higher concentrations and higher pressures, in general, increased the selectivity and reaction rate, while higher temperatures increased the reaction rate but decreased selectivity.

Researchers from Dr. Reddy's Laboratories and Dow Chemical Laboratories jointly investigated the asymmetric hydrogenation of an amino acid precursor en route to **94** for the type II diabetes inhibitor denagliptin.[52] After screening substrates, solvents, and precatalysts, conditions were selected for reduction of tetrasubstituted olefin **92** to **93** (Scheme 7). In early optimization studies, the researchers utilized a DoE for the hydrogenation, focusing on minimizing the charge of the catalyst [(*S*)-PhanePhos Rh COD]BF_4. Using a substrate:catalyst ratio of 500:1, the variables were substrate concentration (20−100 g/L), H_2 pressure (4−12 bar), and reaction temperature (20−50 °C). For these experiments, the chiral induction was fairly constant at 94−96% ee, so conversion was selected as the primary response. The main factor influencing the extent of conversion to **93** was the concentration of **92**, followed by the H_2 pressure. The model predicted the best conversions at high concentration of **92**, high pressure, and high temperature. Subsequent investigations showed that inhibitory impurities were removed by recrystallizing **92**, allowing the hydrogenation to proceed reliably with lower precatalyst charges.

Reductive amination of lactol **95** was used in the manufacture of the fesoterodine intermediate **96**, in a route free of protecting groups (Scheme 8).[53] The primary side products were the over-

Scheme 8. Reductive Amination Using H_2



reduced amine **98** and triol **99**, which probably arose through the quinone methide intermediate **97**. Through DoE studies, the Pfizer (Sandwich) group found that reduction using H_2 and Pd/C gave the highest selectivity, as compared to hydrogenation with other catalysts or reductive amination[54] using $NaCNBH_3$ or $NaBH_4$.

DoE was used to optimize the charges of $NaBH_4$ and BF_3·ether for the diborane-mediated reduction of secondary amide **79** (Scheme 9).[55] In initial studies the mandelate salt was broken by treatment with aq $NaOH/CH_2Cl_2$, and the solvent for the solution of the free base was displaced by THF; subsequent studies showed that 0.2 equiv of nBu_4NBr could be added to dissolve the mandelate salt directly in THF. A CCD was carried out at 55−57 °C to assess the conversion to **100** after 5 h by varying the amount of $NaBH_4$ (1.0, 4.0 equiv) and BF_3·Et_2O (0.5, 3.0 equiv); 10 experiments total with two center points. The optimal conditions were ∼3.5 equiv of $NaBH_4$ and ∼3 equiv of BF_3·Et_2O. To control the diborane emissions, the reduction was carried out in a sealed vessel, and a second CCD was carried out. The results showed that at 55 °C, 90% reduction could be achieved using 3 equiv of $NaBH_4$ and 2.7−3.0 equiv of BF_3·Et_2O, relatively small decreases in the charges of those reagents. For safety considerations, commercially available BF_3·THF was used in the optimized process.

Researchers from Argonaut Technologies used DoE to optimize the preparation of oxazolidinone **103**, beginning with the reduction of L-phenylalanine (**101**) to L-phenylalaninol (**102**) through borane generated from $NaBH_4$ and iodine (Scheme 9).[56] The product was treated with triphosgene to afford **103**. A DoE was employed for the generation of **102**, varying the charges of $NaBH_4$ (1.1−2.0 equiv) and I_2 (0.55−1.0 equiv) and reaction time (1−4 h). Eight experiments were run, along with two replicates. Runs with 0.89 equiv of I_2 gave essentially the same yield as runs with 1.0 equiv, so the substoichiometric charge was employed. The response surface curve for the data showed that times longer than 1 h did not affect the yield and that there was a plateau for the yield between 1.6 and 2.0 equiv of $NaBH_4$. A charge of 1.8 equiv of $NaBH_4$ was chosen to ensure complete reduction within 1 h on scale, and the process was scaled up smoothly.

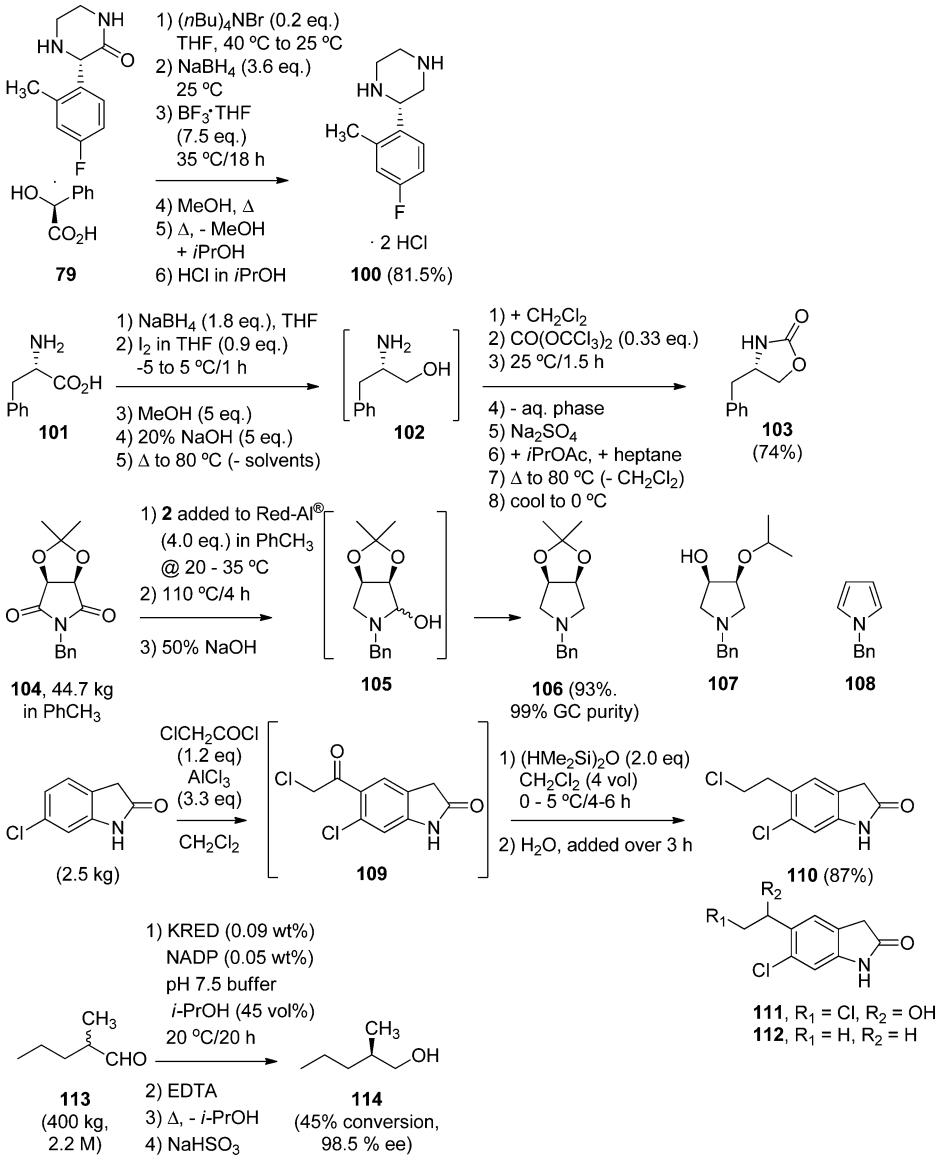
A Pfizer/DSM collaboration applied DoE to develop a scalable synthesis of pyrrolidine **106**, an intermediate[57] in the synthesis of ingliforib, a candidate for treatment of diabetes (Scheme 9). Reduction of imide **104** to amine **106** in toluene with BH_3 generated from $NaBH_4/BF_3$ proceeded in good yield, but generated 3−5% of ether **107**. Red-Al (Na-$(MeOCH_2CH_2O)_2AlH_2$) was not expected to generate **107** and was chosen as a safer and less expensive alternative to BH_3·THF. The initial reaction of Red-Al (3 equiv) at 110 °C did not generate **107** but led to the formation of 27% of aminal **105**, which was found to convert to **106** with time and more Red-Al. With extended reaction times the pyrrole byproduct **108** grew. A fractional factorial design (2^{3-1}) with 2 center point replicates generated only six reactions in studying the effects of time, temperature, and Red-Al charge. The responses measured were the purity of desired **106** and A% of intermediate **105**. No details on the range for each variable were provided, but the desired product purity was strongly correlated with the higher temperature. An inverse addition was used for successful scale-up. Using DoE, the process was rapidly optimized, even before the structure of intermediate **105** had been established.[58]

Pfizer workers[59] used an experimental design approach to gain insight into the reduction of ketone **109** to form the oxindole **110**, an intermediate in the synthesis of ziprasidone hydrochloride (Scheme 9). The reactive chloroketone was known to be a skin sensitizer, so for EHS reasons the Friedel−Crafts acylation was telescoped into the reduction. Reaction of **109** with Et_3SiH generated **110** and the intermediate alcohol **111**, but controlling the amount of over-reduced alkane **112** was difficult. Aluminum chloride-mediated deoxygenation using tetramethyldisiloxane (TMDS) cleanly afforded **110**. TMDS was preferred over polymethylhydrosiloxane, in part because of easier workup. Three factors were studied in the DoE: TMDS charge (2−4 equiv), reaction temperature (0−25 °C), and concentration of **109** in dichloromethane (6−10 vol). A full factorial design (2^3) was employed, plus one center point, for nine reactions. DoE analysis indicated that only the TMDS charge was relevant, but the optimum value was also dependent on the $AlCl_3$ charge from the previous step. This was reported to be the first commercially viable process.

A team of Codexis/Pfizer collaborators investigated the process optimization of the biocatalytic kinetic resolution of racemic 2-methylvaleraldehyde (**113**) to prepare (*R*)-2-methyl-

dx.doi.org/10.1021/op500169m | Org. Process Res. Dev. 2015, 19, 1605−1633

Scheme 9. Reductions



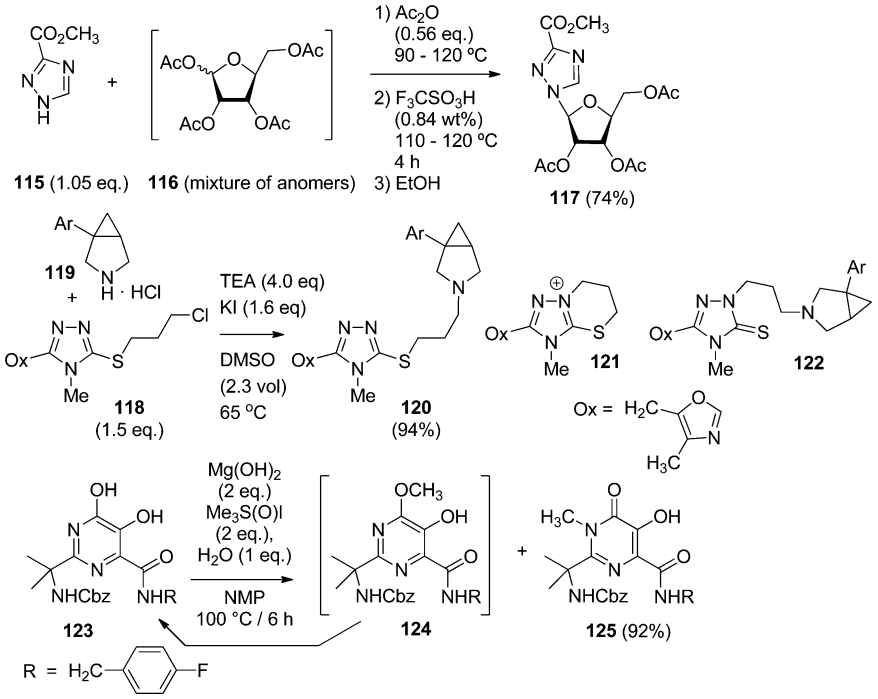
pentanol (**114**; Scheme 9).[60] Reduction was carried out with a ketoreductase (KRED), with *i*-PrOH as the bulk reductant. Three variables were studied in a screening design using two 12-place parallel synthesis carousels: loading of KRED (0.75−2.5 g/L), loading of the cofactor NADP (0.04−0.13 g/L), and reaction temperature (15 °C−25 °C). The substrate concentration (3.5 mL/g), pH (7.4), stirring rate (100 rpm), and % IPA (58% v/v) were kept constant. Two responses, conversion and selectivity (% ee), were measured at 4, 8, and 23 h time points. All three time points showed that the KRED concentration was the most influential factor affecting the conversion, with higher KRED loading leading to higher conversion. Lower temperatures gave higher selectivities. The cofactor concentration had no impact on either the conversion or selectivity, allowing the researchers to decrease the charge of this relatively expensive cocatalyst. On scale, the reduction was quenched by EDTA, and the unreacted aldehydes were removed by codistillation and treatment with $NaHSO_3$.

C−N Bond Formation. Roche researchers used DoE to optimize the formation of a protected nucleoside of L-ribose, **117** (Scheme 10).[61] The mixture of triazole **115** and anomers **116** (in DCM) was treated with Ac_2O to remove water, and the volatiles were removed under reduced pressure. A catalytic amount of triflic acid was added, and after about 4 h the mass was cooled and diluted with EtOH to crystallize **117**. A central-composite DoE was used to assess the influence of temperature (105−135 °C) and charge of TfOH (0.7−3.0 wt %). Optimal conditions included 0.7−1.5 wt % of TfOH and 110−120 °C; at greater charges and higher temperatures, the reaction mass turned dark, and at lower charges and lower temperatures, the reaction was too slow.

A GSK development team[62] developed a robust S_N2 displacement step in the synthesis of analogues of dopamine D_3 receptor antagonist SB-277011. The reaction between chloropropylthiotriazole **118** and amine-HCl salt **119** to provide **120** was plagued by the formation of impurity **121** arising from intramolecular cyclization of **118** (Scheme 10). Impurity **121** was formed in 20−25 A% by HPLC, and the derived impurity **122**, isomeric with **120**, was difficult to purge by crystallization. The group simultaneously employed DoE and kinetic modeling to gain further process understanding, while maximizing selectivity and yield and minimizing unreacted starting materials. A full

dx.doi.org/10.1021/op500169m | Org. Process Res. Dev. 2015, 19, 1605−1633

Scheme 10. C−N Bond Formation



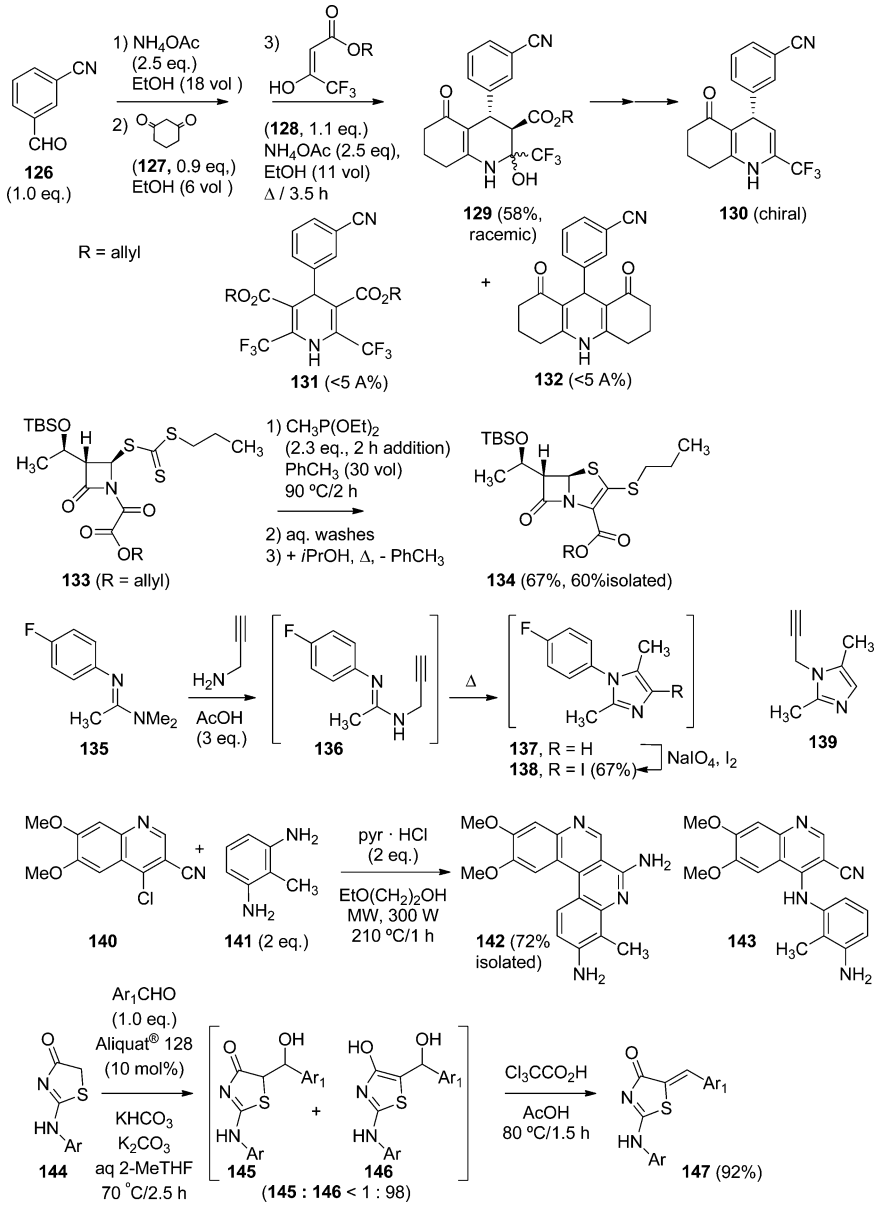
factorial design with two center points (10 reactions) was performed using the Reactarray-SK233 parallel reaction tool. The four factors included the stoichiometries of triazole **118** (1.1−1.6 equiv), triethylamine (2.1−2.6 equiv), and potassium iodide (KI) (0.2−1.6 equiv) and the reaction temperature (50−70 °C). The reaction volume in DMSO was fixed at 4 volumes, the volume of the initial process. The results from the DoE study showed an adequate fit to the data and demonstrated that the high setting for all four factors, which corresponded to the previously existing reaction conditions, led to the highest yield within the design space. In other words, the DoE suggested no improved conditions. Some key observations were derived from the study, namely that the reaction conversion benefits from higher levels of KI, the selectivity benefits from high KI levels and lower temperature, and that higher temperature promotes formation of **121**. A subsequent fractional factorial design, including the DMSO charge (3.6−4.4 volumes), was performed to assess the robustness around the optimal settings from the first design and determine whether more concentrated conditions would encourage the intermolecular alkylation. Unfortunately, the variability of the data implied a lack of robustness around the investigated design space, perhaps because of the stirrability of the thick reaction mixtures. This design, though, did suggest that the selectivity improves at higher concentration (less DMSO). Kinetics studies of the reaction showed that the halogen exchange reaction was the rate-determining step. The pK_A's of amine **119** and TEA are similar, and traditional OFAT studies outside the original design spaces showed that a greater excess of TEA would benefit the formation of the free base of **119**. Another round of factor screening and robustness testing of the optimal factor settings led to the final conditions outlined in Scheme 10 and confirmed a 94% yield with more robust operations, a 6% better yield than the original conditions.

Merck researchers used high-throughput screening and DoE to maximize the *N*-methylation of raltegravir intermediate **123** and reduce solvent usage (Scheme 10).[63] DoE screening was used to examine the effects of reagent stoichiometries, reaction concentration, temperature, and reaction time. *O*-Methylation was a significant side reaction, but the researchers found that with extended reaction time and higher temperature the *O*-methyl impurity **124** was selectively demethylated back to **123**, thereby increasing the ratio of **125** to **124**. Alkylation conditions using MeI and $Mg(OMe)_2$, higher concentration, higher temperature, and more equivalents of reagents favored formation of **125**. To avoid handling the volatile MeI under scale-up conditions, trimethylsulfoxonium iodide was employed, which gradually decomposed to MeI under the reaction conditions. For complete *in situ* demethylation of **124**, at least 1 equiv of H_2O was necessary. The 92% yield from conditions shown in Scheme 10 was a substantial improvement over the initial yield of 70%.

Condensations. An Astra-Zeneca group developed a one-pot, unsymmetrical Hantzch reaction of **126** to provide **129** (60% yield initially)[64] en route to their chiral API candidate **130** (Scheme 11). Four variables were studied: equivalents of dione **127** (0.9−1.3), NH_4OAc (2 × 2.5 to 2 × 3.5), and **128** (0.9−1.3), and solvent charge (20−35 vols). They employed a fractional factorial design (2^{4-1}) with two center points for a total of 10 reactions. The responses were yield of **129** and the amount of tricyclic impurity **132**, originally observed at 15−30%. The reaction concentration and the dione charge had large effects, and they were observed to have an interactive effect as well. The dione charge had a larger impact at higher concentrations than at lower concentrations. But in either instance, a higher dione charge led to a higher yield of **129**, but it also led to formation of higher quantities of impurity **132**. In order to strike a compromise between reaction throughput, maximizing **129** and minimizing **132**, the group settled on the conditions shown in Scheme 11. These changes minimized formation of diester impurity **131** (2−4 A% originally), eliminated the need for chromatography and simplified the isolation. The process was demonstrated to manufacture over one ton of **129** in 58% yield with <0.1% of impurities **131** and **132**.

dx.doi.org/10.1021/op500169m | *Org. Process Res. Dev.* 2015, 19, 1605−1633

Scheme 11. Condensations



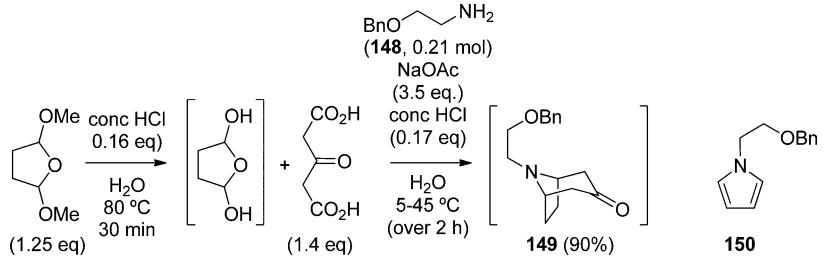
The cyclization of **133** to form thiopenem **134** was optimized using a 2^4 full factorial design with four center points (20 reactions) (Scheme 11). The four factors investigated were reaction temperature (60, 90 °C), concentration (15, 45 volumes), phosphinite stoichiometry (2, 3 equiv), and phosphinite addition time (1, 5 h). The responses quantified were in situ yield at 5 and 21 h. The Pfizer group found that the optimal temperature was about 90 °C, with incomplete reactions at lower temperatures and thermal decomposition at higher temperatures. More dilute reaction conditions were preferred, as expected for an intramolecular reaction. Phosphinite stoichiometry and addition times had relatively little impact on the outcome of this carbene-mediated cyclization. Under optimized conditions, the sulopenem intermediate was isolated in 60% yield, with impurities being effectively purged by crystallization from *i*PrOH. While the yield was only marginally improved over the scouting reaction, the DoE provided the information necessary to reduce the addition time and the time cycle of the manufacturing process.[65]

To optimize the formation of imidazole **137** from amidine **135** (Scheme 11), a group from Roche used DoE to study the effects of propargylamine equivalents, temperature, and time.[66] Eleven experiments were performed, including three center point runs. When more than 1.25 equiv of propargylamine was charged, the yield of **137** dropped off sharply. Using flow NMR, the group found that amine exchange to form **136** occurred without heating, and imidazole **139** was identified as arising from excess propargylamine. Subsequent experiments showed that conc HCl was superior to AcOH, formic acid or TFA, and the preferred conditions using conc HCl were essentially the optimal conditions identified in the DoE. For isolation, **137** was converted to the crystalline iodoimidazole **138**.

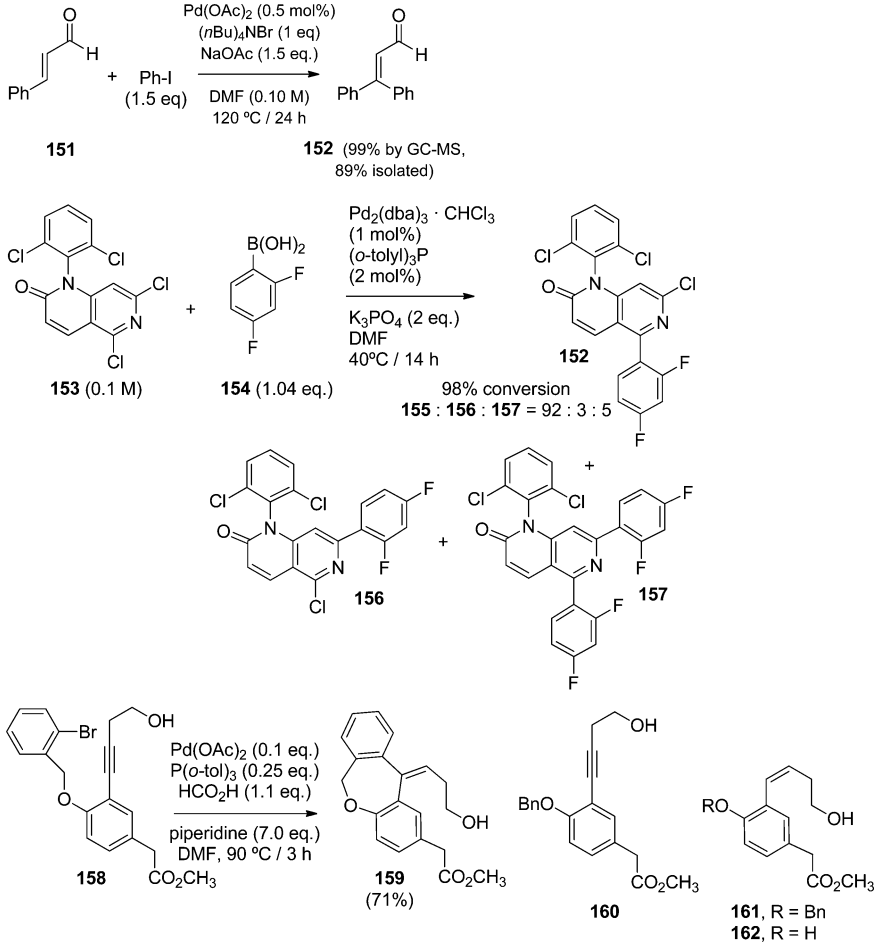
Naphthyridine **143** was found in ~25% yield as a byproduct in the preparation of **142** from **140** and **141** in ethoxyethanol at 130 °C (Scheme 11). When additional **142** was needed for studies, Wyeth researchers used DoE to optimize its formation.[67] Four factors were considered in a full factorial design (2^4 runs plus three center point runs): the charges of **141** (1, 4 equiv) and

dx.doi.org/10.1021/op500169m | *Org. Process Res. Dev.* 2015, 19, 1605−1633

Scheme 12. Robinson Annulation



Scheme 13. Palladium-Mediated Couplings

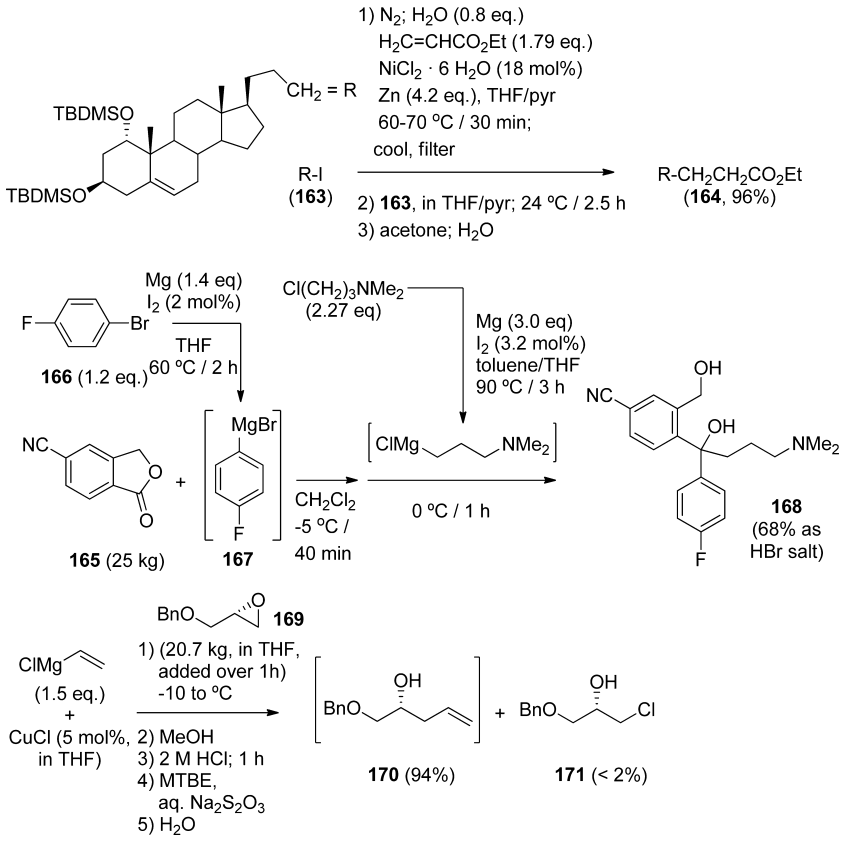


catalyst pyr·HCl (0, 5 equiv), temperature (50, 130 °C), and time (8, 24 h). (Some previous experimentation had shown that reaction concentration had little impact on the formation of **142**.) Analysis of the results clearly showed that higher temperatures and higher charges of **141** and pyr·HCl gave higher yields of **142**, and indicated that the optimal conditions might be outside of those initially chosen. Accordingly, a second DoE was carried out for 8 h at 130 °C, the reflux temperature of the solvent, varying the charges of **141** (1−12 equiv) and pyr·HCl (0−9 equiv). With charges of 9 equiv each of **141** and pyr·HCl, the yield was about 70%, but purification would have been difficult given the large excesses of those components. The researchers found that higher temperatures, accessible in microwave reactors, generated **142** in about 80% yield with considerably lower stoichiometries.

GSK workers[68] sought to optimize the PTC-mediated aldol condensation between an aromatic aldehyde and thiazalone **144** to generate aldol adducts **145** and **146**, which upon acidification yielded olefin **147** (Scheme 11). They employed a D-optimal design to probe the impact of the qualitative factors of solvent (2-BuOH, toluene, 2-MeTHF, MIBK, *i*-PrOAc), Lewis acid (none, $Sc(OTf)_3$, $Y(OTf)_3$, $Yb(OTf)_3$), base (50% NaOH, 2 N NaOH (with and without brine), 50% K_2CO_3, 25% $KHCO_3$), and phase-transfer agent (Aliquat 128, $(n\text{-}Bu)_4NHSO_4$, $BnEt_3NCl$). The values for the following continuous variables were fixed: reactant stoichiometry (1:1), reaction concentration (0.25 M), PTC charge (10 mol %), Lewis acid charge (10 mol %), and reaction temperature (60 °C). The collecting and collation of the HPLC data were accomplished with the iChemExplorer software. The regression analysis revealed that Aliquat 128 and $Yb(OTf)_3$ were beneficial for high conversion, while *i*-PrOAc, 2-MeTHF, and 2-BuOH were the most desirable solvents. They found that the aldol reaction was driven by the crystallization of the enol **146**. Subsequent experiments revealed that pH 11.5 was optimal,

dx.doi.org/10.1021/op500169m | *Org. Process Res. Dev.* 2015, 19, 1605−1633

Scheme 14. Coupling with Grignard Reagents



which led to the use of 1:1 saturated aq $KHCO_3/K_2CO_3$ with 2-MeTHF. $Yb(OTf)_3$ was eventually eliminated, as it had no effect at pH 11.5. Acids with pK_a 0−1.5 were optimal for the dehydration of the **145**/**146** wet cake, and trichloroacetic acid in AcOH was used on scale. Under optimized conditions, olefin **147** was produced in less than 6 h, a marked improvement over the original conditions requiring at least 3 days. Screening was carried out in microreactors with minimal starting materials (3 g) and completed within 2 weeks.

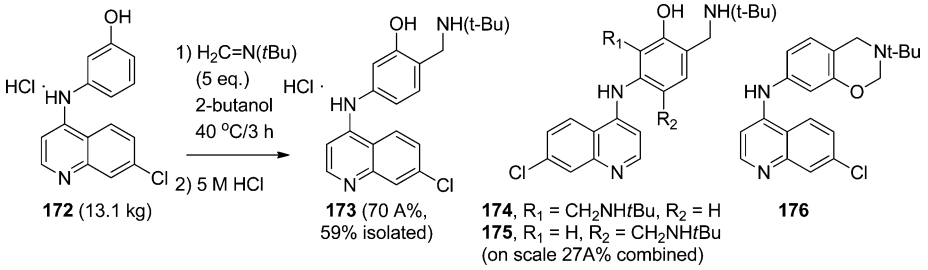
Researchers from GSK used a DoE study to optimize a Robinson annulation to generate tropinone **149** (Scheme 12).[69] Amine **148**, prepared from ethanolamine, was charged as the limiting component. In a total of 10 experiments, seven factors were examined in the DoE: the stoichiometries of the dimethoxy-THF, diacid, hydrated bis-aldehyde, HCl, and NaOAc; the addition temperature; and the rate of heating. The goals were complete consumption of **148** with minimal formation of the pyrrole impurity **150**. Lower charges of the dimethoxy-THF (1.05 equiv) led to incomplete consumption of **148**, while higher charges (1.55 equiv) increased the amount of **150** formed. Reducing the charge of NaOAc from 4.0 equiv to 3.5 equiv was also beneficial in minimizing formation of **150**.

Metal-Catalyzed Cross-Couplings and Metal-Mediated Reactions. Aggarwal and co-workers at the University of Bristol and GSK[70] optimized the Heck coupling between *trans*-cinnamaldehyde (**151**) and iodobenzene (Scheme 13), which had been reported to proceed in only 56% yield (59% conversion). They employed a 2^{5-1} fractional factorial design with 4 center points (20 reactions; 1 mmol scale) to explore the impact of solvent charge (1.6−5.0 mL), temperature (50−90 °C), Pd catalyst loading (0.5−5.0 mol %), NaOAc charge (1.5−4.0 equiv), and reaction time (4−9 h). The analysis revealed that a high temperature was the most relevant setting to attain a high yield of 3,3-diphenylacrylaldehyde (**152**), followed by higher catalyst loading and higher solvent charge; there was a significant interaction between temperature and concentration, which would have been overlooked by OFAT experiments. The NaOAc charge and reaction time had no impact on the yield. Yields in the design space ranged from 5−75%, and curvature was detected from yields at the center point conditions, so another DoE was carried out using an RSM approach. Data from the first run showed that good yields could be achieved using low catalyst charges. The second DoE explored some conditions outside the first DoE: higher reaction temperatures (90−120 °C) and more dilute conditions (5−10 mL). With the exploration of that new design space, the authors included reaction time (8−24 h) and low catalyst loadings. The RSM design, a face-centered central composite (CCD) with 30 experiments, led to improved yields, mainly due to the higher temperature. At the lower Pd loading, high yields could only be obtained at the more dilute conditions (99% yield). The authors rationalized that the higher temperature leads to faster rates and the low Pd loading under higher dilution extends the catalyst lifetime, allowing for full conversion.[71] In 3 weeks the authors improved the reaction yield from 56% to 99%.

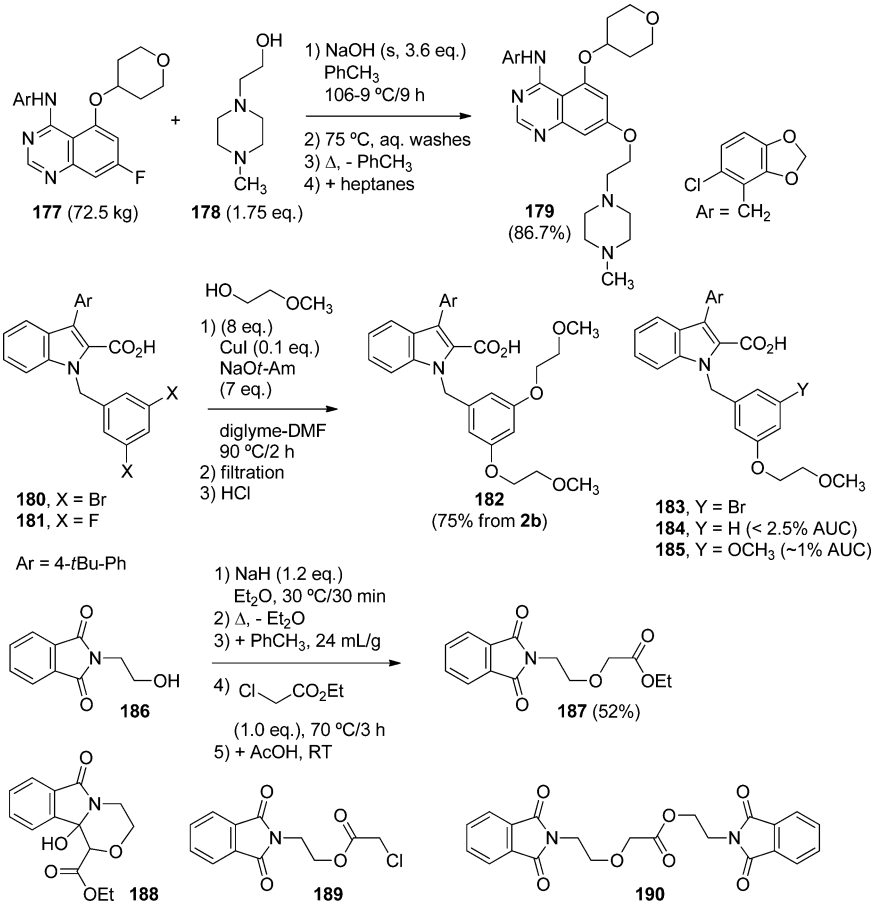
Merck researchers used DoE to optimize a Suzuki coupling[72] following a HTS to identify the optimal solvent, base, and ligand. The design studied temperature (35−65 °C) and the ratio of aryl boronic acid **154** to substrate **153** (0.8 to 1.4), while monitoring conversion, regioselectivity, and yield of **155** (Scheme 13). A central composite design was used, and 13 experiments were run. As expected, higher temperatures gave higher conversions but poor regioselectivity, forming undesired **156** and **157**.

dx.doi.org/10.1021/op500169m | *Org. Process Res. Dev.* 2015, 19, 1605−1633

Scheme 15. Mannich Reaction



Scheme 16. O-Alkylation



Eventually, $(o\text{-tolyl})_3P$ was chosen over other ligands, as it was cheaper in bulk, gave faster conversions, and required less **154**.

In the Pd-catalyzed reductive cyclization of **158** to **159** (Scheme 13), significant amounts of hydrogenolyzed impurities **160**, **161**, and **162** were formed.[73] For optimization, five factors were examined by DoE: equivalents of phosphine ligand (0.1, 0.3), HCO_2H (2.0, 4.0), and piperidine (5.0, 7.0); charge of DMF (10% and 30% v/w); and temperature (50, 90 °C). (The charge of Pd was held constant at 0.1 equiv.) On a 100 mg scale, 17 experiments were run using a half factorial (2^{5-1}) design plus one center point. Higher temperatures and less HCO_2H were found to be critical for good selectivity, as shown by the half-normal plots. Based on this information, the HCO_2H charge was reduced to 1.1 equiv. A follow-up RSM design indicated that the maximum yield was 80%, and the optimal conditions were found to be experimentally robust. Scale-up was expected to be straightforward for these homogeneous reaction conditions.

A team from Hoffman-LaRoche evaluated the reductive coupling between iodide **163** and ethyl acrylate, which affords **164**, a calcitriol precursor (Scheme 14).[74] The robustness of the process was a concern for scale-up, as no recourse was available if the catalyst was not regenerated successfully. Water content was expected to be critical for turnover of the Ni(0) catalyst, and high charges of ethyl acrylate were expected to afford lower yields due to polymerization. Zinc and $NiCl_2{\cdot}6H_2O$ were handled in a glovebag to preclude any deactivation of the catalyst by O_2. A full factorial design with three variables and one center point (9 reactions) was chosen. The variables were additional water (3−6 equiv relative to $NiCl_2{\cdot}6H_2O$), ethyl acrylate (1.5−2.2 equiv), and the $NiCl_2{\cdot}6H_2O$ charge (0.125−0.25 equiv). The only factor in the DoE study that significantly affected the yield was the ethyl acrylate charge, which was then set at 1.85 equiv. Yields were reproducible and approaching quantitative, with ethyl acrylate charges of 1.85 equiv (±5%). Through DoE, the optimized process was found to be fairly robust over the entire design space.

dx.doi.org/10.1021/op500169m | Org. Process Res. Dev. 2015, 19, 1605−1633

Using DoE, a group from Dr. Reddy's Laboratories and the Institute of Science and Technology optimized the process to diol **168**, a key intermediate in the synthesis of citalopram.[75,76] The one-pot route involves formation of two Grignard reagents (including **167**) followed by their sequential reactions, starting with benzolactone **165** (Scheme 14). The full-factorial screening design of the first step studied the amounts of **166** (1.2−1.5 equiv vs **165**), Mg (1.2−1.5 equiv vs **165**), and iodine initiator (1−10 wt %). The resulting 12 reactions (including 4 center points) were assayed for yield, purity and residual **165**. Similarly, the second Grignard reagent was optimized with respect to equivalents of 1-chloro-3-(dimethylamino)propane (1.8−3.0), Mg (2.0−4.0) and iodine (1−10 wt %). Both of the designs were augmented with additional eight reactions to generate a RSM design. Based on the assay yield and purity requirements (>75% and 98%, respectively), design spaces showing the acceptable operating limits were generated indicating a wide range of acceptable conditions. Three validation experiments were then performed to corroborate the model predictions and showed excellent agreement between the prediction and the actual outcome.

A group from Merck (U.K.) sought to optimize the copper-catalyzed reaction of epoxide **169** with commercially available vinyl magnesium chloride (Scheme 14).[77] On initial scale-up they observed that up to 20 A% of chlorohydrin **171** was formed, due to prolonged Grignard addition time. By applying an inverse addition mode, the levels of **171** were suppressed (<1%). A DoE was undertaken to gain further insight into the robustness of this reaction. A 2^{3-1} fractional factorial design with two center points (10 reactions) was performed in THF with CuCl (20 mol %) to examine the effects of temperature (−20 to +20 °C), Grignard stoichiometry (1.5−2.5 equiv), and epoxide addition time (15−105 min). The response monitored was formation of **170**, which ranged from 0.2 to 40% over the design space. The reaction temperature was the dominant factor affecting the outcome, while the other two factors had minimal impact. The reactions run at −20 °C gave lower levels of the chlorohydrin. Additional univariate optimization revealed that the reaction temperature could be raised to −5 °C under the conditions shown in Scheme 14. By quenching with MeOH and then HCl, emulsions and solids were avoided, and the $Na_2S_2O_3$ wash reduced the level of Cu in the product.

Aryl Alkylation. Carbogen researchers[78] optimized the final step in the synthesis of antimalarial agent GSK369796 (**173**), via a Mannich reaction on phenol **172**, initially performed in MeOH (Scheme 15). Driving the reaction to completion was important, as unreacted **172** precipitated from alcoholic reaction solvents and complicated workups. Byproducts were the bis-Mannich adducts **174** and **175** and the benzoxazine **176**, which formed with extended reactions. DoE studies in *i*-PrOH or 2-butanol, chosen for improved product stability, showed that more dilute conditions (35 vol), low reaction temperature (40 °C), and 5 equiv of the Schiff base raised the yield of **173**. After Mannich reaction in 2-butanol and acidification to isolate **173**, impurity **176** was found to be soluble, so the optimized process used 2-butanol as solvent, avoiding chromatographic purification. These improvements raised the isolated yield from ∼30% to 46% with a purity increase from ∼80 A% to 99.9 A%.

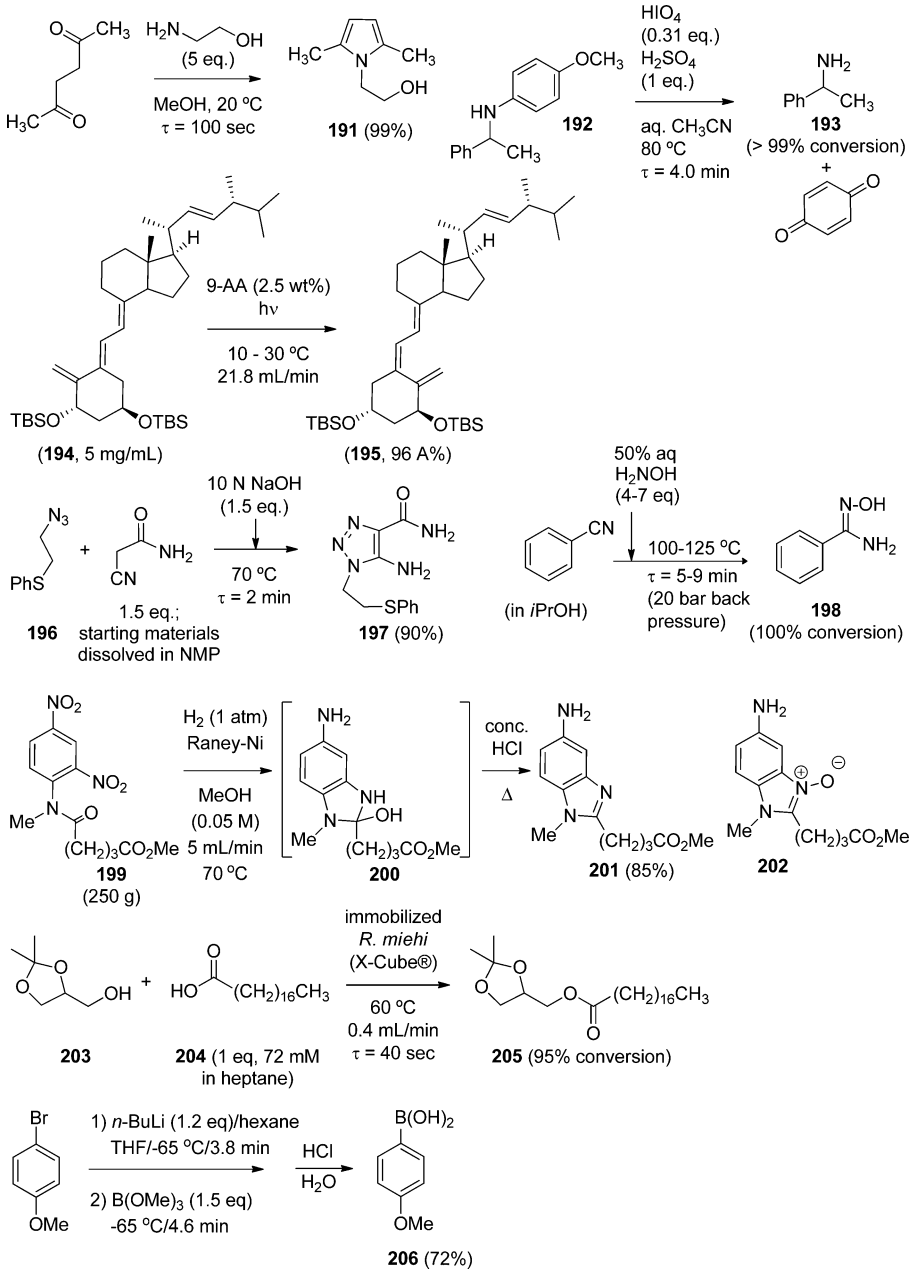
O-Alkylation. An S_NAr displacement of **177** was the last step in the manufacture of API **179** (Scheme 16).[79] The goals of the optimizations were to increase the isolated yield and provide more robust processing conditions, while changing the solvent from *t*-AmOH to toluene. Two levels of four variables were examined: equivs of base and alcohol **178**, H_2O charge, and temperature. A fractional factorial design DoE was run for a total of 10 experiments (2^{4-1} + 2 center points). The AstraZeneca researchers found that more than two equiv of base were not necessary and that charging more equiv of H_2O than base decreased the rate of reaction, by hydrating the base. Increasing the reaction temperature or the charge of alcohol **178** increased the rate of reaction, but with the drawbacks of generating more impurities or increasing the overall cost, respectively. Process optimization raised the yield of **179** from 63% to almost 87%.

Researchers at GSK employed DoE screening to optimize the preparation of API **182** from dibromo intermediate **180** (Scheme 16).[80] Under metal-free conditions, the initial substitution to **183** was rapid, but conversion to **182** required extended reaction times; hence, metal-catalyzed conditions were studied to shorten the overall reaction time. A mixture of DMF-diglyme was chosen as the solvent, as some DMF was necessary for the copper-catalyzed reactions and diglyme was inert under the reaction conditions. The factors varied were the charges of CuI (0.1, 0.7 equiv), NaO*t*Am (6, 7 equiv), and methoxyethanol (8, 10 equiv); temperature (80−90 °C); and time (2−16 h), while screening for short reaction times, the yield of product **182**, and minimal formation of the hydrodebrominated side product **184**. (Impurity **184** was problematic because it was not completely purged from **182** in routine crystallizations; impurity **185** arose from reaction of MeOH, formed by decomposition of methoxyethanol under the basic conditions.) On a 50 L scale, using the optimized conditions, the yield of **182** was 75%, with <0.1 ppm of Cu in isolated **182**. Due to the concerns about minimizing the concentration of copper in the workup streams and controlling the amount of **184** formed, research on **180** was discontinued in favor of reactions with the bis-fluoro intermediate **181**, which did not need metal catalysis.

The alkylation of phthalimidoylethanol **186** with ethyl chloroacetate was examined by DoE (Scheme 16).[81] The alkoxide of **186** was generated in Et_2O using an excess of NaH, and the ether solvent was displaced with toluene. In the first investigations, the factors examined were temperature (24, 70 °C), concentration (24, 48 mL/g), and reaction time (3, 27 h); a total of 11 experiments were run, a full factorial design (2^3) plus 3 center point runs. The highest yield of **187** was obtained by extended reaction at a high temperature under dilute conditions, with the latter probably serving to decrease the formation of the transesterification side product **190**. (Compounds **188** and **189** were mentioned as potential side products.) Subsequent investigations did not increase the yield beyond the 40% achieved from the first DoE. As the reaction mixtures darkened with extended reactions, a second DoE screen was performed, holding experiments at 70 °C over 3 h. The factors examined were charges of NaH (1, 1.2 equiv) and ethyl chloroacetate (1, 1.2 equiv), sonication (none, 1 h), and the presence of catalytic KI (none, 5 mol %) or catalytic *n*-Bu_4NBr (none, 5 mol %). A fractional factorial screen was set up comprising 11 experiments: 2^{5-2} + 3 center point runs. Analysis showed that the small excess of NaH raised the yield, but the addition of the phase-transfer catalyst lowered the yield and increased byproduct formation; the other factors had no effect. Through these investigations, the yield of **187** was raised from 25% to 52%.

Flow Chemistry. The value of microreactors for quickly optimizing conditions has been noted,[82] and approaches to making large quantities of material under continuous flow conditions have been reviewed.[83] With continuous flow reactors, it is necessary to optimize the average residence time, τ, which is

Scheme 17. Flow Chemistry



analogous to the reaction time in batch operations; for this reason, continuous flow reactions have been grouped together.

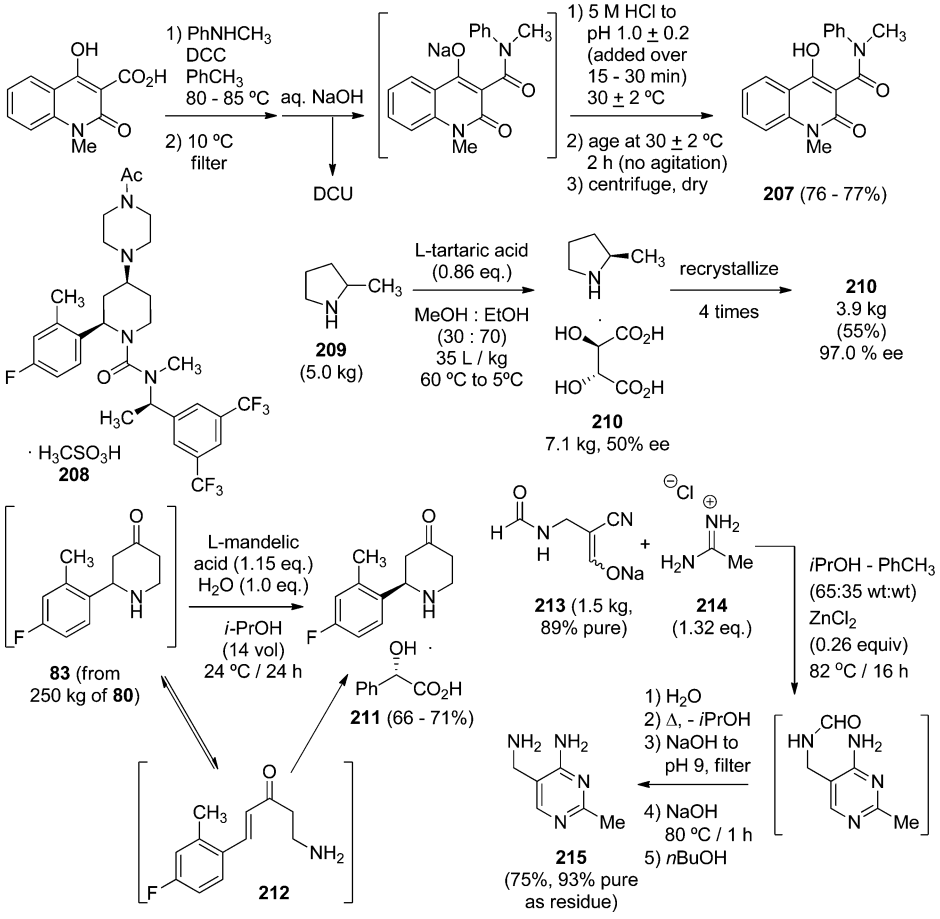
In optimizing the preparation of pyrrole **191** from a Paal–Knorr reaction (Scheme 17) using microreactors, a Dutch group used DoE, focusing on complete conversion with minimal reaction time.[84] They reported four levels each of stoichiometry and length of reaction, with three levels for reaction temperature. A total of 58 experiments were run, with an average run time of only 25 s. Essentially, a quantitative yield was achieved, without an acid catalyst.

Microreactors were used to screen conditions to remove a *para*-methoxyphenyl (PMP) group in amine **192**, using HIO_4/H_2SO_4 (Scheme 17).[85] Using a D-optimal screening design, 51 reactions were carried out in less than 6 h, consuming approximately 10 mg of **192**. Data showed that high temperatures gave faster conversion to **193** (>99% conversion at 90 °C/1.3 min), but in a larger microreactor, the boiling solvents (H_2O, acetonitrile) produced an unsteady flow. Accordingly, a reaction temperature of 80 °C was successfully adopted.

Genzyme researchers sought to optimize the continuous photoisomerization of **194** to **195** (Scheme 17), an intermediate in the synthesis of doxercalciferol, a treatment for chronic kidney disease.[86] For efficient conversion they used a continuous photochemical reactor, with solutions of **194** being pumped through tubing wrapped around a photolysis cell. They conducted a 2^4 full factorial design looking at the effects of temperature (10–30 °C), flow rate (5.5–21.8 mL/min), substrate concentration in heptane (5.0–50.0 mg/mL), and the photosensitizer 9-acetylanthracene (9-AA, 4–16 wt %). This approach with 3 center points led to 19 reactions, measuring A% of **195** as the output. A full factorial design was employed because only a few minutes were required to run 100 mg reactions and isolate the crude product. Conversions varied from 62–96% over the design space. The goals were to test the robustness around

dx.doi.org/10.1021/op500169m | Org. Process Res. Dev. 2015, 19, 1605–1633

Scheme 18. Crystallization and Resolution



the center point values and to minimize the use of 9-AA, which was anticipated to be troublesome to remove from **195**. Data analysis showed that temperature and 9-AA charge had little bearing on the outcome of the reaction; thus, the charge for the latter was set to 4 wt %. The flow rate and concentration interacted, with high conversions at high concentration/low flow rates and low concentrations/high flow rates. Subsequent work showed that the 9-AA charge could be lowered to 2.5 wt % and was removed by continuous filtration through a bed of carbon and Celite. Overall, the 96 A% outcome was the same as the original conditions, but the 9-AA charge was reduced from 10 to 2.5 wt % and the process was demonstrated to be robust over a wide temperature range.

Wyeth researchers employed DoE to optimize the preparation of aminotriazole **197** (Scheme 17).[87] Due to safety concerns, continuous operations were selected, and the β-azidoethyl phenyl sulfide **196** was chosen as a safer alternative to ethyl azide. In the DoE study, the factors varied were temperature (65−95 °C), and charges of cyanoacetamide (1.5, 2 equiv) and NaOH (1.5, 2 equiv). In this design, the 16 experiments were carried out monitoring the formation of **197** and the amount of unreacted cyanoacetamide. Yields of **197** fell off at higher temperatures due to the side reaction of NaOH with cyanoacetamide.

Hungarian co-workers applied continuous flow technology to the reaction of benzonitrile with 50% hydroxylamine to afford the corresponding amidoxime (**198**; Scheme 17), a precursor to oxadiazoles.[88] Hydroxylamine is known to decompose exothermically with the generation of gases, and the temperature for thermal decomposition is lowered significantly in the presence of metal ions. A microreactor was chosen for process optimization due to the inherently greater safety from heating only small quantities of reactants at any moment, and because the glass surfaces of the microreactor were essentially free of iron and other metals. Optimized conversions were probed looking at the effects of reaction temperature (75−125 °C), H_2NHOH stoichiometry (1−7 equiv), and residence time (1−9 min) using a central composite design (19 reactions). Data analysis with the MODDE software revealed high conversions (>97%) could be attained over a wide range of factor settings with a 5 min residence time, namely at 125 °C with 4 equiv of H_2NHOH or at 100 °C with 7 equiv of H_2NHOH. The DoE runs were performed with a 10 μL reactor, and these optimized conditions worked smoothly in a 450 μL reactor.

Teva process chemists applied fractional factorial design to identify the critical process parameters in the continuous flow synthesis of benzimidazole **201** (via **200**), an intermediate in the synthesis of bendamustine hydrochloride (Scheme 17).[89] The original process, a batch mode hydrogenation of bis-nitro arene **199**, generated high amounts of the N-oxide impurity **202** through incomplete reduction. Batch hydrogenation also posed safety concerns, and specialized reactors would be required on scale to maintain a hydrogen overpressure and to dissipate the heat from the very exothermic reduction of **199**. Continuous flow reactors offer advantages through ready heat transfer and efficient mixing; with the H-Cube continuous flow hydrogenator, hydrogen is generated on demand by electrolysis of H_2O.[90] Initial continuous flow hydrogenation was developed using the

dx.doi.org/10.1021/op500169m | Org. Process Res. Dev. 2015, 19, 1605−1633

H-Cube with Raney-Ni as the catalyst and methanol as solvent. Using the maximum rate of generating H_2 by the H-Cube, optimization of the flow conditions focused on the following parameters: temperature (20−100 °C), system pressure (1−50 bar), flow rate (0.3−3.0 mL/min), and concentration of **199** in MeOH (0.01−0.06 M). Higher concentrations were not attainable due to limited solubility of **4** in MeOH. The nine reaction study (1 center point) was analyzed with *Design-Expert* software and revealed that the concentration and flow rate were the most impactful factors. The other two factors, temperature and pressure, had little or no impact on yield. With low concentration (<0.025 M) and low flow rates (<0.5 mL/min), the conversion was high (>95%). The optimal conditions (0.02 M, 0.4 mL/min flow at 50−70 °C and 1−5 bar) resulted in a 97% conversion, consistent with the DoE model prediction. Using data from the DoE for the H-Cube and exploratory experiments with the H-Cube Midi, five hydrogenations were scaled up with the H-Cube Midi using the conditions shown in Scheme 17.

de Souza and co-workers applied DoE to optimize a biocatalytic conversion by continuous flow through an immobilized enzyme in an X-Cube reactor.[91] The esterification of (*R,S*)-1,2-isopropylidine glycerol (**203**) with stearic acid (**204**) was catalyzed by immobilized lipase from *Rhizomucor miehi*, providing **205**, a precursor to a monoacyl glycerol (Scheme 17). Initial optimization provided 87% conversion at 60 °C at 0.6 mL/min. A 2^{3-1} fractional factorial design (3 center points; 7 reactions) examined the effects of temperature (40−60 °C), substrate concentration (35−100 mM), and flow rate (0.2−3.0 mL/min), and analysis showed that only temperature had no bearing on the outcome. Thus, temperature was fixed at 60 °C in a subsequent RSM design (3 center points; 11 reactions). As the first design suggested that generally lower flow rates and higher concentrations were beneficial, the ranges in the RSM design were 0.4−0.8 mL/min and 72−95 mM, respectively. The RSM showed that yields fell off sharply from the optimal conditions.

A University of Bergen team developed a multijet oscillating disk minireactor system for performing flow chemistries at cryogenic temperatures. They employed DoE to optimize the continuous synthesis of phenylboronic **206** acid via the lithiation of 4-bromoanisole and quenching with methyl borate (Scheme 17).[92] Preliminary experiments had indicated that total residence time and temperature influenced the reaction yield, which was maximized at 71%. A two-factor full factorial design with two center points led to six experiments over the ranges of −70 to −60 °C and 7.3 to 10.95 min for combined average residence times. As expected, both variables were relevant, as was the two-factor interaction term, suggesting that, at the warmer temperatures, shorter reactions were favored, while, at colder temperatures, longer reaction times led to higher yields. In an effort to increase the yield, they extrapolated outside the design space, into a region that suggested that at −75 °C and 10.9 min total residence time that >90% yields could be attained. A reaction at those conditions provided a 69% yield, probably because other variables were not considered. In principle, an output of over 2 kg/day could be achieved under these conditions.

Crystallization and Resolution. A team from DuPont Chemoswed required batches of Roquinimex (**207**, Scheme 18) to have uniform physical properties to afford a suitable dissolution profile (>90% after 1 h).[93] Crude amide **207** was prepared by a coupling with dicyclohexylcarbodiimide (DCC) and purified by filtering off residual dicyclohexylurea (DCU) from a solution of the sodium salt. To crystallize **207**, 5 M HCl was added to pH 1. A distinct relationship between the particle size and good dissolution was detected with larger crystals. A 2^3 full factorial design with 3 center points (11 reactions) of the crystallization variables was performed by studying the influence of temperature (20−30 °C), acid dosing period (15−30 min), and concentration (6−11 volumes water). The single response was the dissolution rate (% after 1 h) and ranged from 63−93% over the design space. The data showed that larger crystals were produced at higher crystallization temperatures and with longer dosing times, as expected. All three factors were statistically relevant, with the higher aging temperature being the most impactful, followed by longer dosing time and lower dilution. A relevant interaction between concentration and dosing time indicated that, at shorter dose times, more dilute conditions were preferred but at the longer dose period, the more concentrated solution gave an improved dissolution profile. The DoE-optimized crystallization was successfully performed on three 40 kg batches and provided **207** with dissolution rates of 94−95% (vs 77−93% prior to DoE). By controlling the crystallization, the levels of residual *N*-methylaniline were controlled and the yields improved from 68−72% to 76−77%.

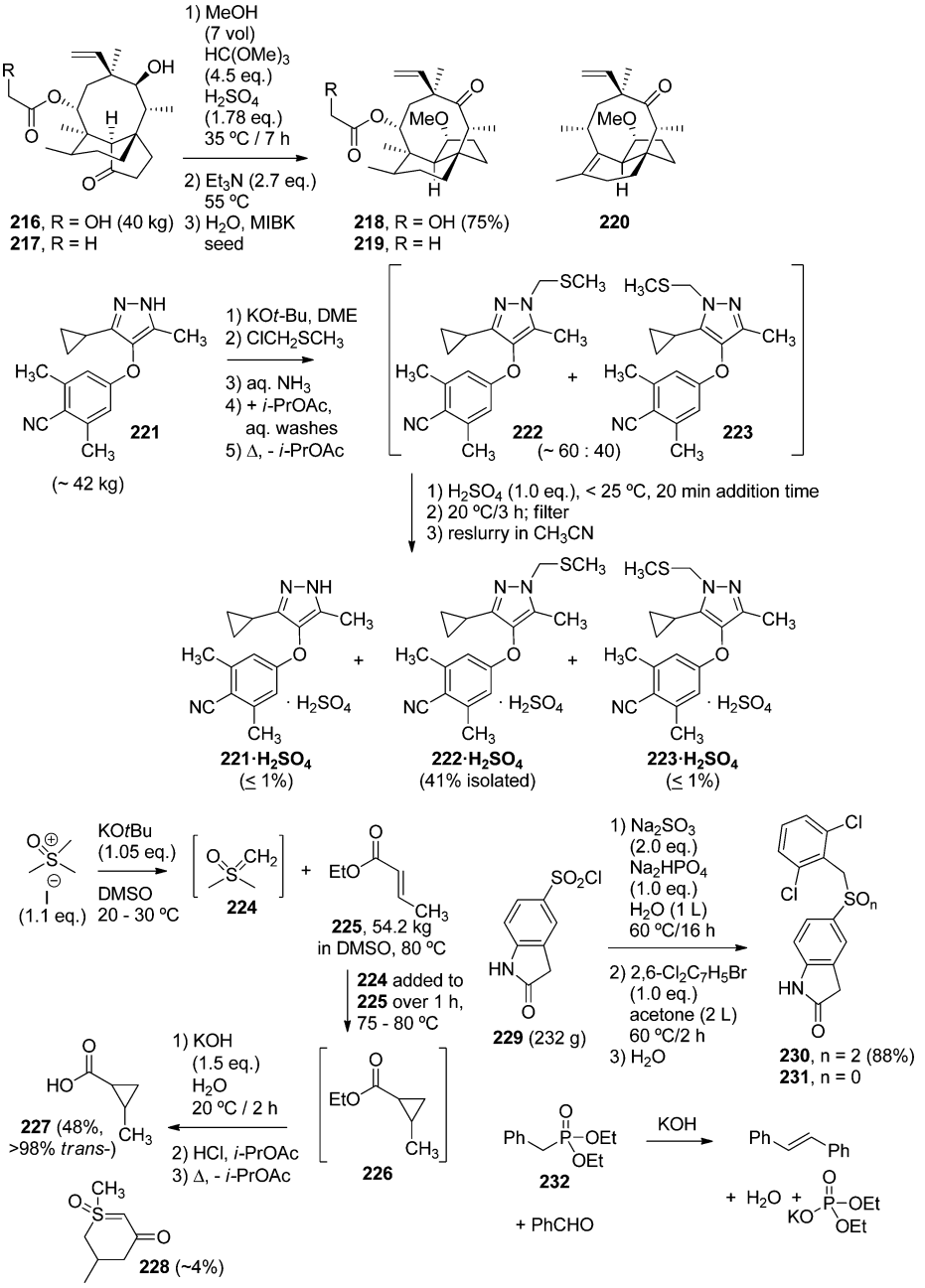
Form 1 of the NK_1 antagonist casopitant mesylate (**208**) was developed by GSK initially, but late in development it was discovered that the API was actually a mixture of Forms 1 and 3. Competitive slurry experiments showed crystallization of Form 1 was favored below 20 °C and that of Form 3 was favored above 30 °C (i.e., enantiotropic), but under no conditions did one form crystallize exclusively. Careful analysis of the preclinical toxicology batch revealed it contained 27% w/w Form 3; thus, this became the upper limit for future batches.[94] As part of a QbD approach to defining the design space for the crystallization of the API, they applied DoE to develop a robust process, defined as a mixture of polymorphs with the Form 3 content capped at 27%. The first design screened eight continuous variables (35 reactions): acetone (3.5−5.5 vol) and EtOAc (3.0−5.0 vol), stirring power (4−320 W/m^3), seed amount (0.25−0.75 wt %), seeding temperature (30−50 °C), age time before isooctane addition (0.5−2.5 h), isooctane added (2−4 vol), and isooctane addition time (0.5−1.5 h). Three different batch purities were included in the study (low, standard, and modified) by spiking the crystallizations with known impurities.

A second DoE (10 reactions) studied a narrower range of those factors found to be relevant from the first design in order to define the proven acceptable ranges (PARs). The combined data sets indicated that seeding temperature was the most impactful factor, followed by the seeding amount. Higher seeding temperatures and seeding with smaller amounts of Form 1 led to higher levels of undesired Form 3. The remaining parameters had little or no impact on the formation of Form 3. Even under the most unfavorable conditions within the design space, the undesired form was present at no more than 14 w/w%, well below the threshold value. The robustness of the crystallization led to the conclusion that the Form 3 level in the API was not a critical quality attribute (CQA).

Abbott researchers used DoE to optimize conditions for the resolution of **209** (Scheme 18).[95] Three factors were considered: the tartaric acid equivalents, solvent volume, and isolation temperature. Four successive recrystallizations raised the ee of salt **210** from 50% to 97% for eventual conversion to the API.

As part of a QbD approach to control genotoxic impurities in a fluoroarylamine (structure not shown), a team from GSK employed DoE to optimize the rejection of mesylate esters resulting from isolating the product as the MSA salt.[96] Under the optimal crystallization conditions that had been chosen, the

Scheme 19. Miscellaneous Reactions



moisture content was low (≤0.4 wt %) and an excess of methanesulfonic acid relative to the free base was charged; those conditions favor the formation of mesylate esters.[97] At the time of these investigations, the carcinogenicity potency of ethyl mesylate was not available. The impacts of the following parameters were studied: seeding temperature (34−44 °C), volume of EtOAc (3.0−5.0), volume of acetone (4.2−4.8), volume of isooctane (2.5−3.5), and isooctane addition time (0.5−1.5 h). A fractional factorial (2^{5-2}) design was used in a 10 reaction study, including two center points. The measured responses were the residual levels of the methyl, ethyl, and isopropyl mesylate esters in the API, likely arising from the corresponding alcohols being present from upstream processing. In all cases, <1 ppm of the mesylate esters was found in both the product suspensions and the isolated salts. The robustness of the operations throughout isolation and drying was also demonstrated, permitting the argument that testing batches of the mesylate salt for these mesylate esters would be unnecessary.

A key step in the synthesis of GSK's casopitant mesylate, the CIDR on racemic amine **83** to produce (*R*)-amine salt **211**, was optimized with DoE (Scheme 18).[98,99] Epimerization of the methine carbon probably proceeds through an acid-catalyzed retro-Michael/Michael equilibrium with amine **212**. Water was added to minimize the formation of three impurities arising from self-condensation of two molecules of **212**, which dramatically lowered the isolated yield. The investigators studied the impact of the reaction time, temperature, water equiv (0.5−1.5), solvent volumes (IPA; 11−14), and L-mandelic acid stoichiometry (0.9−1.3) on the yield of **211** and the formation of the unwanted diastereomeric amine salt. The formation of the unwanted diastereomer was kept <1.5% with lower amounts of mandelic acid, water, and the reaction volume. The yield ranged from 47−

dx.doi.org/10.1021/op500169m | Org. Process Res. Dev. 2015, 19, 1605−1633

63% over the design space and was favored by longer reaction time, lower temperature, and less mandelic acid. To preclude racemization during slow filtrations on scale, residual *i*-PrOH in the wet cake was displaced with cyclohexane. A subsequent DoE to confirm the robustness over the proposed proven acceptable range (PAR) was run and confirmed with scoping experiments at forcing, mild, and midpoint settings for the relevant parameters.

DSM chemists used DoE to optimize the $ZnCl_2$-catalyzed condensation of enolate **213** with amidinium chlorides to produce 4-aminopyrimidines (Scheme 18), used in the industrial synthesis of vitamin B_1.[100] Having settled on an IPA/toluene solvent mixture, the team observed several runs to suffer from reaction mixtures that stuck to the vessel walls and were difficult to stir, and predicting which mixtures would be stirrable was not straightforward. Accordingly, they screened the following three factors to maximize the yield of pyrimidine **215** while ensuring stirrability: (1) % *i*PrOH in toluene (65−90%), (2) acetamidine (**214**) charge (0.95−1.40 equiv), and (3) $ZnCl_2$ charge (0.15−0.30 equiv). The yields ranged from 68 to 80% over the design space. Ultimately, a DoE model, using STAVEX 5.1, was obtained (although not disclosed) that resulted in a higher R^2 value of 0.96. The optimized process was demonstrated on an cubic meter scale.

Pfizer researchers used DoE to simplify an amidation−amination sequence in the synthesis of CB_1 antagonist, CP-945,598-01. The large number of process variables for the extractive workup of the amide intermediate led the researchers to develop a direct isolation instead. They also used DoE studies to select the proper solvent composition (30% water/70% THF) and seed temperature (45 °C) for the crystallization of the penultimate aminated species.[101]

Miscellaneous Reactions. GSK researchers employed DoE to optimize the acid-catalyzed rearrangement of pleuromutilin, **216** (Scheme 19).[102] Initially, conditions had to be sufficiently robust to tolerate variable amounts of acetate impurity **217** found in batches of the natural product **216**. A half-factorial study was carried out to maximize the consumption of **216** (target <3% unreacted), by varying temperature (20, 40 °C), charges of MeOH (2, 6 vol), H_2SO_4 (0.1, 0.5 vol), and trimethylorthoformate (TMOF, 0.6, 2 vol). Analysis of the data showed that the behavior was considerably nonlinear and that the optimum might lie outside of the ranges initially chosen. A second DoE was conducted using a central composite design, focusing on maximizing conversion to **218** and minimizing the formation of the major impurity, alkene **220** (target <4%). Four factors were examined: temperature (10−40 °C) and the charges of MeOH (0−40 mL/g), H_2SO_4 (0.25−1.0 mL/g), and TMOF (0.4−1.6 mL/g); 30 experiments were carried out, including six center point runs. An interaction between acid equiv and temperature was most influential such that, at 10 °C, the amount of alkene was low (<0.5%) and constant over the range of acid equiv; while, at 30 °C, the alkene level was universally higher (4 to 6.4%) but dependent on the acid amount. The optimal conditions were initially performed at 25 °C (40 kg pilot scale) and gave yields up to 79% with >97% purity. In order to reduce time cycles from 24 to 6 h, acceptable results were achieved at 35 °C with no loss in purity.

Prompted by an unexpected 10% yield loss during the synthesis of **222·H_2SO_4** on scale (Scheme 19), a Pfizer group used DoE to minimize decomposition to **221** via loss of the methylthiomethyl group.[103] In the initial process work up, the postquench mixture of regioisomers **222** and **223** in *i*-PrOAc was treated with H_2SO_4 at 50 °C, aged for 12 h at 20−50 °C, filtered, and reslurried in acetonitrile. The variables examined included the H_2SO_4 charge (0.8, 1.2 equiv), salt formation temperature (30, 70 °C), H_2O charge (0.05, 0.4%), and H_2SO_4 addition time (5, 25 min). Ten experiments were run: 2^{4-1} plus two runs at the center point setting. Temperature was found to be the most critical factor, with increased degradation to **221·H_2SO_4** seen with increasing temperature or with extended hold time before filtration. Based on the DoE analysis, the salt was isolated after stirring at 20 °C for 3 h, which served to reduce the level of **221·H_2SO_4** by up to 50%. In the plant under the optimized conditions, two batches (42 kg input) gave an improved yield (41% vs 37%) and acceptable quality of **222·H_2SO_4** for the current scale-up needs.

Lilly researchers used DoE to optimize the cyclopropanation of olefin **225** with dimethylsulfoxonium methylide (Corey's ylide, **224**; Scheme 19).[104] Five factors were examined: temperature (60, 80 °C), cation (Na^+ or K^+), anion (*t*-BuO^-, HO^-), addition type (base or preformed **224**), and addition time (40, 80 min). A total of 16 experiments were run (2^{5-1}, no center points). Higher yields of **226** were favored by using *t*-BuOK to preform ylide **224**, with the 40 min addition at 80 °C. Eventually, the researchers found that the highest yield was obtained at 95 °C with a 15 min addition time, but these conditions were avoided due to safety concerns for scale-up. To avoid isolating the volatile **226**, hydrolysis to acid **227** was telescoped with the cyclopropanation. The major impurity, **228**, was removed by the extractive workup.

Researchers from Pharmacia and SUGEN employed DoE studies in the early stage optimization of a reductive sulfonylation (Scheme 19).[105] Reductions of the sulfonyl chloride **229** to the sulfinate intermediate (not shown) were carried out with zinc dust or sodium sulfite. Initial scale-up of Zn(0)-mediated reductions gave lower yields of **230** than anticipated, probably due to the agglomeration of the zinc dust, and as much as 5 A% of the sulfide side product **231** was formed. By resorting to reduction with Na_2SO_3, the researchers anticipated that the reaction mixture would be homogeneous and that over-reduction would be unlikely. The alkylation of the sulfinate intermediate was found to be robust, so attention was turned to DoE optimization of the reduction. Three factors were considered initially: Na_2SO_3 charge (2, 4 equiv), temperature (60, 100 °C), and time (6, 24 h) (full factorial plus one duplicated run to assess reproducibility for a total of 9 runs). Markedly lower yields were found when a greater charge of Na_2SO_3 was used, and the higher-temperature reaction was extended. The conditions that gave a 93% yield of **230** on screening with a 1 mmol charge of **229** produced **230** in only 70% when scaled up to 10 mmol; poor mass transfer of the biphasic (liquid−liquid) reaction mass was suspected. A second DoE screen was set up to assess whether poor mass transfer was causing low yields, and eight factors were considered: concentration (0.25, 0.5 M), charge of sulfite (2.0, 2.5 equiv), temperature (60, 80 °C), time for reduction (6, 24 h), cation (Na^+, K^+), presence or absence of a phase-transfer catalyst, reaction time (1, 2 h), and solvent (DMF, acetone). For the second screen, 17 experiments were run (2^{8-4} plus a center point): a low-resolution design due to limited resources. The authors concluded that none of the main effects that could influence mass transfer (concentration, reaction time, use of PTC, e.g.) were significant, implicating an isolated occurrence of poor mixing due to magnetic stirring in the 10 mmol run with the low yield (*vide supra*). Scale-up of the biphasic reaction mass was carried out using vigorous mechanical agitation. Overall, using

Na_2SO_3, the yield of **230** was improved from 40−45% to 88%, and impurity **231** was not observed.

DoE was used to analyze a Horner−Emmons reaction between benzaldehyde and diethyl benzylphosphonate, **232** (Scheme 19).[106] The variables screened were base addition period (10, 20 min), initial temperature (5, 40 °C), and final temperature (40, 80 °C). High yields were obtained when both temperatures were low and the addition time was long, or when the temperatures were high and the addition time was short. Additional experiments showed that the reaction was second-order in benzaldehyde and that the competing reaction was hydrolysis of **232**. For best control of the olefination on scale and to minimize hydrolysis of **232**, the authors recommended carrying out the reaction at a lower temperature.

Analytical Investigations. Researchers in France used DoE to calculate the specific heat capacity (C_P) of solvents through analyses in a RC1 reaction calorimeter.[107] The accuracy of these values is important to calculate the adiabatic temperature increase of reaction mixtures. Using a central composite design, 20 experiments were carried out on three solvents, which included six center point runs to assess the reproducibility. The factors examined were the experimentally determined heat capacity, the volume of solvent charged to the calorimeter, and the agitation rate. Analysis showed that the greatest error between the experimentally determined C_P and the true value of C_P occurred with low volumes in the reactor and high agitation rate. A model was created that analysts can apply to reaction calorimeters in their lab.

Researchers in the Netherlands analyzed intermediates in a steroidal contraceptive that had been manufactured for 20 years.[108] The API was made in more than 10 steps from a plant-derived starting material, and intermediates had been assayed by TLC. In order to comply with QbD directives and to preclude failing batches of intermediates by relatively subjective TLC analyses, the researchers developed an ultrahigh-performance liquid chromatography (UHPLC) method for the last five steps. A DoE study was set up to assess the robustness of the analytical method, by examining performance under conditions that bracketed the optimized conditions. The factors examined were column temperature (30, 50 °C), mobile phase flow rate (0.7, 0.9 mL/min), and composition of the mobile phase (92%, 98% acetonitrile in Eluent B). A full factorial study with three center point runs was set up, for a total of 11 runs; responses were peak resolution and assay times. The assay method was found to be robust and suitable for the last five steps. Not only were TLC assays replaced by a less subjective UHPLC assay, but assay times for the intermediates could be reduced to as little as 25% of the TLC assay time.

CONCLUSION

DoE is gaining wider acceptance as another valuable tool for process optimization in the pharmaceutical industry. When experimentation and analyses are reproducible, the time required for generating insightful data may be cut in half or decreased even further. A review of publications in *Organic Process Research & Development* that used DoE shows that it has been implemented at various stages of process development. As Aggarwal and Owen noted, "The models generated by both the screening and the optimization studies are approximations of reality. They are used to predict the favored settings and then these settings can be implemented in order to validate the model."[70] The process chemist and engineer must apply their experience to ensure that both the parameters chosen for the DoE and the results are practicable, reproducible, and relevant to the overall project objectives. We can expect that DoE will be used increasingly for efficient process development.

ASSOCIATED CONTENT

Supporting Information

Bibliographic information for the publications on DoE in OPRD that appeared from 2003−2013, used for Figure 1. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Authors

*E-mail: sweissman@jstar-research.com. Phone: 908-791-9811 (x520).
*E-mail: nganderson@wildblue.net. Phone: 541-899-5555.

Author Contributions

The manuscript was written through contributions of both authors. Both authors have given approval to the final version of the manuscript.

Funding

No funds were used to support the research of this manuscript.

Notes

The authors declare no competing financial interest.

Biographies

Steven A. Weissman, a native of Marblehead, MA, received his B.A. degree in Chemistry from the University of Vermont in 1983 and his Ph.D. from Tufts University in the field of organophosphorus chemistry. He then accepted a postdoctoral appointment with Professor Hebert C. Brown (Purdue University) before joining the Merck Manufacturing division in 1990. In 1997, he moved to Merck Process Research, where he rose to the rank of Senior Investigator and was responsible for developing practical syntheses of pre-clinical candidates, as well as contributed to the commercial routes for Crixivan, Isentress, and Emend. In 2010, he joined Concert Pharmaceuticals as their head of Process Chemistry, and in 2013, he joined J-Star Research as a Director, where he advises clients on process characterization strategies through DoE investigations to facilitate regulatory filings and chemical development. Dr. Weissman has authored over 30 peer-reviewed publications and been cited as an inventor on 11 U.S. patents.

Neal G. Anderson received his B.Sc. degree in Honors Biology from the University of Illinois in 1972 and his Ph.D. in medicinal chemistry from the University of Michigan in 1977. After an industrial post-doc, he joined E. R. Squibb & Sons in 1979. Working in chemical process R&D, he developed and implemented processes for scale-up of many drug candidates and intermediates, along with processes to manufacture aztreonam, captopril, fosinopril sodium, and nadolol. After 17 years, he left Bristol-Myers Squibb as a Principal Scientist. Since 1997 he has consulted internationally, lectured, and presented short courses on chemical process R&D of "small molecules". He is the author or co-author of 16 peer-reviewed publications and one book chapter, and he is an inventor on six U.S. patents. He is the author of *Practical Process Research & Development—A Guide for Organic Chemists* (Academic Press; 2nd edition, 2012).

■ ABBREVIATIONS

ANOVA, analysis of variance; CCD, central composite design; CCF, central composite face-centered design; CIDR, crystallization-induced dynamic resolution; DoE, design of experiments; Fm, 9-fluorenylmethyl; HTS, high-throughput screening; KRED, ketoreductase; QbD, quality by design; OFAT, one factor at a time; OVAT, one variable at a time; RSM, response surface methodology

■ REFERENCES

(1) In this review, DoE includes "design of experiments", "design of experiment", "response surface methodology", and "factorial experimental design".

(2) Laird, T. *Org. Process Res. Dev.* **2002**, *6*, 337.

(3) Mills, J. E. Design of Experiments in Pharmaceutical Process Research and Development. In *Chemical Process Research*; Abdel-Magid, A. F., Ragan, J. A., Eds.; ACS Symposium Series 870; American Chemical Society: Washington, DC, 2003; Chapter 6, pp 87−109.

(4) Mullin, R. Design of Experiments Makes A Comeback. *Chem. Eng. News* **2013**, April 1, pp 25−28.

(5) In 2002, it was stated that delaying the introduction of a product by six months could reduce the lifetime profit by 50%: Ritter, S. K. *Chem. Eng. News* **2002**, *80* (47), 19.

(6) For example, the FDA now provides the breakthrough NDA filing designation to sponsors for fast track or accelerated approvals and priority review. For more information, see http://www.fda.gov/Drugs/DevelopmentApprovalProcess.

(7) Anderson, N. G.; Burdick, D. C.; Reeve, M. M. *Org. Process Res. Dev.* **2011**, *15*, 162.

(8) (a) ICH Harmonized Tripartite Guideline Q11, Development and Manufactrue of Drug Substances (Chemical Entities and Biotechnological/Biological Entities), May 2012: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q11/Q11_Step_4.pdf (accessed 4/24/2014). (b) For instance, Vertex researchers used DoE to advance a QbD filing for a hydrogenation: Looker, A. R.; Ryan, M. P.; Neubert-Langille, B. J.; Naji, R. *Org. Process Res. Dev.* **2010**, *14*, 1032.

(9) Leahy, D. K.; Tucker, J. L.; Mergelsberg, I.; Dunn, P. J.; Kopach, M. E.; Purohit, V. C. *Org. Process Res. Dev.* **2013**, *17*, 1099.

(10) Andraos, J. *Org. Process Res. Dev.* **2005**, *9*, 149.

(11) Lendrem, D.; Owen, M.; Godbert, S. *Org. Process Res. Dev.* **2001**, *5*, 324.

(12) (a) Pilipauskas, D. R. Using Factorial Experiments in the Development of Process Chemistry. In *Process Chemistry in the Pharmaceutical Industry*; Gadamasetti, K. G., Ed.; Dekker: New York, 1999. (b) *Statistics for Experimenters*; Box, G. E. P.; Hunter, W. G.; Hunter, J. S.; Wiley: New York, 1978. (c) Carlson, R.; Carlson, J. E. *Design and Optimization in Organic Synthesis*; Elsevier: Amsterdam, 2005.

(13) For instance, Pfizer researchers determined through DoE the optimal catalyst and solvents to minimize hydrogenolysis byproducts from the reductive amination used to manufacture sertraline: Taber, G. P.; Pfisterer, D. M.; Colberg, J. C. *Org. Process Res. Dev.* **2004**, *8*, 385.

(14) Kuethe, J. T.; Tellers, D. M.; Weissman, S. A.; Yasuda, N. *Org. Process Res. Dev.* **2009**, *13*, 471.

(15) Bacchi, S.; Yahyah, M.; Carangio, A.; Ribecai, A.; Oliveros, M. C. C. *Org. Process Res. Dev.* **2010**, *14*, 332. This reference provides an example of a more complex design using D-Optimal design.

(16) *Continuous factors* are those variables to which a numerical value can be assigned (temperature, stoichiometry, e.g.) in contrast to *discreet factors* (solvent, ligand, reagent, base, e.g.).

(17) The center point is the mean value of the high and low settings for a given variable/factor.

(18) Optimization designs are frequently synonymous with response surface models (RSM) and central composite designs.

(19) Only a handful of non-OPRD papers on the topic were published in this time frame. Although they could have been included, we chose to limit our discussion to those from this journal.

(20) An overview of the application of DoE to optimization of an HPLC method can be found here: Reid, G. L.; Morgado, J.; Barnett, K.; Harrington, B.; Wang, J.; Harwood, J.; Fortin, D. Analytical Quality by Design (AQbD) in Pharmaceutical Development. *Am. Pharm. Rev.* published online: Aug. 27, 2013. http://www.americanpharmaceuticalreview.com/Featured-Articles/144191-Analytical-Quality-by-Design-AQbD-in-Pharmaceutical-Development/ (accessed June 25, 2014).

(21) The optimized conditions are detailed in the schemes, rather than in the body of the text.

(22) Hobson, L. A.; Akiti, O.; Deshmukh, S. S.; Harper, S.; Katipally, K.; Lai, C. J.; Livingston, R. C.; Lo, E.; Miller, M. M.; Ramakrishnan, S.; Shen, L.; Spink, J.; Tummala, S.; Wei, C.; Yamamoto, K.; Young, J.; Parsons, R. L., Jr. *Org. Process Res. Dev.* **2010**, *14*, 441.

(23) DelMonte, A. J.; Fan, Y.; Girard, K. P.; Jones, G. S.; Waltermire, R. E.; Rosso, V.; Wang, X. *Org. Process Res. Dev.* **2011**, *15*, 64.

(24) Mobele, B. I.; Venkatraman, S.; McNaughton-Smith, G.; Gibb, C.; Ulysse, L. G.; Lindmark, C. A.; Shaw, S.; Marron, B.; Spear, K.; Suto, M. J. *Org. Process Res. Dev.* **2012**, *16*, 1385.

(25) Banks, A.; Breen, G. F.; Caine, D.; Carey, J. S.; Drake, C.; Forth, M. A.; Gladwin, A.; Guelfi, S.; Hayes, J. F.; Maragni, P.; Morgan, D. O.; Oxley, P.; Perboni, A.; Popkin, M. E.; Rawlinson, F.; Roux, G. *Org. Process Res. Dev.* **2009**, *13*, 1130.

(26) Hajmowicz, H.; Wisialski, J.; Synoradzki, L. *Org. Process Res. Dev.* **2011**, *15*, 427.

(27) Weiberth, F. J.; Gill, H. S.; Jiang, Y.; Lee, G. E.; Lienard, P.; Pemberton, C.; Powers, M. R.; Subotkowski, W.; Tomasik, W.; Vanasse, B. J.; Yu, Y. *Org. Process Res. Dev.* **2010**, *14*, 623.

(28) Manoel, E. A.; Pais, K. C.; Cunha, A. G.; Simas, A. B. C.; Coelho, M. A. Z.; Freire, D. M. G. *Org. Process Res. Dev.* **2012**, *16*, 1378.

(29) Guercio, G.; Bacchi, S.; Perboni, A.; Leroi, C.; Tinazzi, F.; Bientinesi, I.; Hourdin, M.; Goodyear, M.; Curti, S.; Provera, S.; Cimarosti, Z. *Org. Process Res. Dev.* **2009**, *13*, 1100.

(30) Cimarosti, Z.; Bravo, F.; Castoldi, D.; Tinazzi, F.; Provera, S.; Perboni, A.; Papini, D.; Westerduin, P. *Org. Process Res. Dev.* **2010**, *14*, 805.

(31) Guercio, G.; Perboni, A.; Tinazzi, F.; Rovatti, L.; Provera, S. *Org. Process Res. Dev.* **2010**, *14*, 840.
(32) Roemmele, R. C.; Christie, M. A. *Org. Process Res. Dev.* **2013**, *17*, 422.
(33) Argentine, M. D.; Braden, T. M.; Czarnik, J.; Conder, E. W.; Dunlap, S. E.; Fennell, J. W.; LaPack, M. A.; Rothhaar, R. R.; Scherer, R. B.; Schmid, C. R.; Vicenzi, J. T.; Wei, J. G.; Werner, J. A.; Roginski, R. T. *Org. Process Res. Dev.* **2009**, *13*, 131.
(34) Weiberth, F. J.; Yu, Y.; Subotkowski, W.; Pemberton, C. *Org. Process Res. Dev.* **2012**, *16*, 1967.
(35) Goodman, S. N.; Dai, Q.; Wang, J.; Clark, W. M., Jr. *Org. Process Res. Dev.* **2011**, *15*, 123.
(36) Barton, B.; Logie, C. G.; Schoonees, B. M.; Zeelie, B. *Org. Process Res. Dev.* **2005**, *9*, 70.
(37) Hida, T.; Fukui, Y.; Kawata, K.; Kabaki, M.; Masui, T.; Fumoto, M.; Nogusa, H. *Org. Process Res. Dev.* **2010**, *14*, 289.
(38) Hida, T.; Nogusa, H. *Tetrahedron* **2009**, *65*, 270.
(39) Quittmann, W.; Roberge, D. M.; Bessard, Y. *Org. Process Res. Dev.* **2004**, *8*, 1036.
(40) Rossi, F.; Corcella, F.; Caldarelli, F. S.; Heidempergher, F.; Marchionni, C.; Auguadro, M.; Cattaneo, M.; Ceriani, L.; Visentin, G.; Ventrella, G.; Pinciroli, V.; Ramella, G.; Candiani, I.; Bedeschi, A.; Tomasi, A.; Kline, B. J.; Martinez, C. A.; Yazbeck, D.; Kucera, D. J. *Org. Process Res. Dev.* **2008**, *12*, 322.
(41) In glass-lined vessels, the reaction of Deoxo-Fluor with the glass, as evidenced by the observance of pitting, is surmised to produce a fluorosilicate, which is known to be beneficial to this type of reactions (see Radhamani, K. N.; Padma, D. K. *J. Fluorine Chem.* **1993**, 95).
(42) Hanselmann, R.; Johnson, G.; Reeve, M. M.; Huang, S.-T. *Org. Process Res. Dev.* **2009**, *13*, 54.
(43) Moseley, J. D.; Moss, W. O.; Welham, M. J.; Ancell, C. L.; Banister, J.; Bowden, S. A.; Norton, G.; Young, M. J. *Org. Process Res. Dev.* **2003**, *7*, 58.
(44) Bescond, P.; Graindorge, H.; Mace, H. EP91337A1, 1999. Bescond, P.; Graindorge, H.; Mace, H. US 5,973,149, 1999.
(45) Bayat, Y.; Hajimirsadeghi, S. S.; Pourmortazavi, S. M. *Org. Process Res. Dev.* **2011**, *15*, 810. The hexabenzyl precursor to TADB was prepared from glyoxal and benzylamine.
(46) Bayat, Y.; Ebrahimi, H.; Fotouhi-Far, F. *Org. Process Res. Dev.* **2012**, *16*, 1733.
(47) Guercio, G.; Bacchi, S.; Goodyear, M.; Carangio, A.; Tinazzi, F.; Curti, S. *Org. Process Res. Dev.* **2008**, *12*, 1188.
(48) (a) Anderson, N. G. *Org. Process Res. Dev.* **2005**, *9*, 800. (b) Brands, K. M. J.; Davies, A. J. *Chem. Rev.* **2006**, *106*, 2711.
(49) Bravo, F.; Cimarosti, Z.; Tinazzi, F.; Castoldi, D.; Stonestreet, P.; Galgano, A.; Westerduin, P. *Org. Process Res. Dev.* **2010**, *14*, 832.
(50) Alorati, A. D.; Gibb, A. D.; Mullens, P. R.; Stewart, G. W. *Org. Process Res. Dev.* **2012**, *16*, 1947.
(51) Chaplin, D.; Harrison, P.; Henschke, J. P.; Lennon, I. C.; Meek, G.; Moran, P.; Pilkington, C. J.; Ramsden, J. A.; Watkins, S.; Zanotti-Gerosa, A. *Org. Process Res. Dev.* **2003**, *7*, 89.
(52) Appell, R. B.; Boulton, L. T.; Daugs, E. D.; Hansen, M.; Hanson, C. H.; Heinrich, J.; Kronig, C.; Lloyd, R. C.; Louks, D.; Nitz, M.; Praquin, C.; Ramsden, J. A.; Samuel, H.; Smit, M.; Willets, M. *Org. Process Res. Dev.* **2013**, *17*, 69.
(53) Dirat, O.; Bibb, A. J.; Burns, C. M.; Checksfield, G. D.; Dillon, B. R.; Field, S. E.; Fussell, S. J.; Green, S. P.; Mason, C.; Mathew, J.; Mathew, S.; Moses, I. B.; Nikiforov, P. I.; Pettman, A. J.; Susanne, F. *Org. Process Res. Dev.* **2011**, *15*, 1010.
(54) Another reductive amination was discussed with Scheme 3.
(55) Guercio, G.; Manzo, A. M.; Goodyear, M.; Bacchi, S.; Curti, S.; Provera, S. *Org. Process Res. Dev.* **2009**, *13*, 489. In the optimized process, $(n\mathrm{Bu})_4\mathrm{NBr}$ was added to solubilize the mandelate salt in THF, avoiding the salt split and some racemization that had been produced by the presence of residual NaOH that was extracted into the organic phase.
(56) Vo, L.; Ciula, J.; Gooding, O. W. *Org. Process Res. Dev.* **2003**, *7*, 514.
(57) Alimardanov, A. R.; Barrila, M. T.; Busch, F. R.; Carey, J. J.; Couturier, M. A.; Cui, C. *Org. Process Res. Dev.* **2004**, *8*, 834.
(58) Other intermediates have been proposed and monitored by React-IR in the reduction of a succinimide to the corresponding pyrrolidine: see ref 15.
(59) Nadkarni, D. V.; Hallissey, J. F. *Org. Process Res. Dev.* **2008**, *12*, 1142.
(60) Gooding, O. W.; Voladri, R.; Bautista, A.; Hopkins, T.; Huisman, G.; Jenne, S.; Ma, S.; Mundorff, E. C.; Savile, M. M.; Truesdell, S. J.; Wong, J. W. *Org. Process Res. Dev.* **2010**, *14*, 119.
(61) Zhang, P.; Dong, Z. E.; Cleary, T. P. *Org. Process Res. Dev.* **2005**, *9*, 583.
(62) Massari, L.; Panelli, L.; Hughes, M.; Stazi, F.; Maton, W.; Westerduin, P.; Scaravelli, F.; Bacchi, S. *Org. Process Res. Dev.* **2010**, *14*, 1364.
(63) Humphrey, G. R.; Pye, P. J.; Zhong, Y.-L.; Angelaud, R.; Askin, D.; Belyk, K. M.; Maligres, P. E.; Mancheno, D. E.; Miller, R. A.; Reamer, R. A.; Weissman, S. A. *Org. Process Res. Dev.* **2011**, *15*, 73.
(64) Hopes, P. A.; Parker, A. J.; Patel, I. *Org. Process Res. Dev.* **2006**, *10*, 808.
(65) Brenek, S. J.; Caron, S.; Chisowe, E.; Delude, M. P.; Drexler, M. T.; Ewing, M. D.; Handfield, R. E.; Ide, N. D.; Nadkarni, D. V.; Nelson, J. D.; Olivier, M.; Perfect, H. H.; Phillips, J. E.; Teixeira, J. J.; Weekly, R. M.; Zelina, J. P. *Org. Process Res. Dev.* **2012**, *16*, 1348.
(66) Barrios Sosa, A. C.; Williamson, R. T.; Conway, R.; Shankar, A.; Sumpter, R.; Cleary, T. *Org. Process Res. Dev.* **2011**, *15*, 449.
(67) Gopalsamy, A.; Shi, M.; Nilakantan, R. *Org. Process Res. Dev.* **2007**, *11*, 450.
(68) Corona, J. A.; Davis, R. D.; Kedia, S. B.; Mitchell, M. B. *Org. Process Res. Dev.* **2010**, *14*, 712.
(69) Bream, R. N.; Hayes, D.; Hulcoop, D. G.; Whiteman, A. J. *Org. Process Res. Dev.* **2013**, *17*, 641.
(70) Aggarwal, V. K.; Staubitz, A. C.; Owen, M. *Org. Process Res. Dev.* **2006**, *10*, 64.
(71) These conditions of high temperature, low catalyst loadings, and relatively dilute conditions are analogous to the optimal conditions for other Pd-catalyzed couplings without ligands: (a) de Vries, A. H. M.; Mulders, J. M. C. A.; Mommers, J. H. M.; Henderickx, H. J. W.; de Vries, J. G. *Org. Lett.* **2003**, *5*, 3285. (b) Arvela, R. K.; Leadbeater, N. E.; Sangi, M. S.; Williams, V. A.; Granados, P.; Singer, R. D. *J. Org. Chem.* **2005**, *70*, 161.
(72) Cai, C.; Chung, J. Y. L.; McWilliams, J. C.; Sun, Y.; Shultz, C. S.; Palucki, M. *Org. Process Res. Dev.* **2007**, *11*, 328.
(73) Nishimura, K.; Kinugawa, M. *Org. Process Res. Dev.* **2012**, *16*, 225.
(74) Van Arnum, S. D.; Moffet, H.; Carpenter, B. K. *Org. Process Res. Dev.* **2004**, *8*, 769.
(75) Vedantham, R.; Vetukuri, V. N. K. V. P. R.; Boini, A.; Khagga, M.; Bandichhor, R. *Org. Process Res. Dev.* **2013**, *17*, 798.
(76) For a review of escitalopram syntheses, see: Harrington, P. J. *Pharmaceutical Process Chemistry for Synthesis; Rethinking the Routes to Scale-Up*; Wiley-VCH; 2011; pp 30−91.
(77) Alam, M.; Wise, C.; Baxter, C. A.; Cleator, E.; Walkinshaw, A. *Org. Process Res. Dev.* **2012**, *16*, 435.
(78) Lawrence, R. M.; Dennis, K. C.; O'Neill, P. M.; Hahn, D. U.; Roeder, M.; Struppe, C. *Org. Process Res. Dev.* **2008**, *12*, 294.
(79) Raw, S. A.; Taylor, B. A.; Tomasi, S. *Org. Process Res. Dev.* **2011**, *15*, 688.
(80) Bullock, K. M.; Burton, D.; Corona, J.; Diederich, A.; Glover, B.; Harvey, K.; Mitchell, M. B.; Trone, M. D.; Yule, R.; Zhang, Y.; Toczko, J. F. *Org. Process Res. Dev.* **2009**, *13*, 303.
(81) Synoradzki, L.; Jańczewski, D.; Włostowski, M. *Org. Process Res. Dev.* **2005**, *9*, 18.
(82) Kirschning, A. *Beilstein J. Org. Chem.* **2013**, *9*, 1696.
(83) Anderson, N. G. *Org. Process Res. Dev.* **2012**, *16*, 852.
(84) Nieuwland, P. J.; Segers, R.; Koch, K.; van Hest, J. C. M.; Rutjes, F. P. J. T. *Org. Process Res. Dev.* **2011**, *15*, 783.
(85) Koch, K.; van Weerdenburg, B. J. A.; Verkade, J. M. M.; Nieuwland, P. J.; Rutjes, F. P. J. T.; van Hest, J. C. M. *Org. Process Res. Dev.* **2009**, *13*, 1003.
(86) Anderson, B. G.; Bauta, W. E.; Cantrell, W. R., Jr. *Org. Process Res. Dev.* **2012**, *16*, 967.

(87) Tinder, R.; Farr, R.; Heid, R.; Zhao, R.; Rarig, R. S., Jr.; Storz, T. *Org. Process Res. Dev.* **2009**, *13*, 1401.
(88) Vörös, A.; Baán, Z.; Mizsey, P.; Finta, Z. *Org. Process Res. Dev.* **2012**, *16*, 1717.
(89) Chen, J.; Przyuski, K.; Roemmele, R.; Bakale, R. P. *Org. Process Res. Dev.* **2013**, DOI: 10.1021/op400179f.
(90) http://thalesnano.com/products/h-cube (accessed 11 April 2014).
(91) Junior, I. I.; Flores, M. C.; Sutili, F. K.; Leite, S. G. F.; de M. Miranda, L. S.; Leal, I. C. R.; de Souza, R. O. M. A. *Org. Process Res. Dev.* **2012**, *16*, 1098.
(92) Sleveland, D.; Bjørsvik, H.-R. *Org. Process Res. Dev.* **2012**, *16*, 1121.
(93) Sjövall, S.; Hansen, L.; Granquist, B. *Org. Process Res. Dev.* **2004**, *8*, 802.
(94) Cimarosti, Z.; Castagnoli, C.; Rossetti, M.; Scarati, M.; Day, C.; Johnson, B.; Westerduin, P. *Org. Process Res. Dev.* **2010**, *14*, 1337.
(95) Pu, Y.-M.; Grieme, T.; Gupta, A.; Plata, D.; Bhatia, A. V.; Cowart, M.; Ku, Y.-Y. *Org. Process Res. Dev.* **2005**, *9*, 45.
(96) Cimarosti, Z.; Bravo, F.; Stonestreet, P.; Tinazzi, F.; Vecchi, O.; Camurri, G. *Org. Process Res. Dev.* **2010**, *14*, 993.
(97) (a) Teasdale, A.; Eyley, S. C.; Delaney, E.; Jacq, K.; Taylor-Worth, K.; Lipczynski, A.; Reif, V.; Elder, D. P.; Facchine, K. L.; Golec, S.; Oestrich, R. S.; Sandra, P.; David, F. *Org. Process Res. Dev.* **2009**, *13*, 429. (b) Teasdale, A.; Delaney, E. J.; Eyley, S. C.; Jacq, K.; Taylor-Worth, K.; Lipczynski, A.; Hoffmann, W.; Reif, V.; Elder, D. P.; Facchine, K. L.; Golec, S.; Oestrich, R. S.; Sandra, P.; David, F. *Org. Process Res. Dev.* **2010**, *14*, 999.
(98) Bravo, F.; Cimarosti, Z.; Tinazzi, F.; Smith, G. E.; Castoldi, D.; Provera, S.; Westerduin, P. *Org. Process Res. Dev.* **2010**, *14*, 1162.
(99) Another CIDR, using 3,5-dichlorosalicylaldehyde, is discussed with Scheme 7.
(100) Létinois, U.; Schütz, J.; Härter, R.; Stoll, R.; Huffschmidt, F.; Bonrath, W.; Karge, R. *Org. Process Res. Dev.* **2013**, *17*, 427.
(101) Ragan, J. A.; Bourassa, D. E.; Blunt, J.; Breen, D.; Busch, F. R.; Cordi, E. M.; Damon, D. B.; Do, N.; Engtrakul, A.; Lynch, D.; McDermott, R. E.; Mongillo, J. A.; O'Sullivan, M. M.; Rose, P. R.; Vanderplas, B. C. *Org. Process Res. Dev.* **2009**, *13*, 186.
(102) Andemichael, Y.; Chen, J.; Clawson, J. S.; Dai, W.; Diederich, A.; Downing, S. V.; Freyer, A. J.; Liu, P.; Oh, L. M.; Patience, D. B.; Sharpe, S.; Sisko, J.; Tsui, J.; Vogt, F. G.; Wang, J.; Wernersbach, L.; Webb, E. C.; Wertman, J.; Zhou, L. *Org. Process Res. Dev.* **2009**, *13*, 729.
(103) de Koning, P. D.; McManus, D. J.; Bandurek, G. R. *Org. Process Res. Dev.* **2011**, *15*, 1081.
(104) Delhaye, L.; Stevens, C.; Merschaert, A.; Delbeke, P.; Briône, W.; Tilstam, U.; Borghese, A.; Geldhof, G.; Diker, K.; Dubois, A.; Barberis, M.; Casarubios, L. *Org. Process Res. Dev.* **2007**, *11*, 1104.
(105) Chen, J. J.; Nugent, T. C.; Lu, C. V.; Kondapally, S.; Giannousis, P.; Wang, Y.; Wilmot, J. T. *Org. Process Res. Dev.* **2003**, *7*, 313.
(106) Grabarnick, M.; Zamir, S. *Org. Process Res. Dev.* **2003**, *7*, 237.
(107) Alozie, D.-M.; Courtes, P.; Ha, B.; Prat, D. *Org. Process Res. Dev.* **2011**, *15*, 1412.
(108) Musters, J.; van den Bos, L.; Kellenbach, E. *Org. Process Res. Dev.* **2013**, *17*, 87.