The Evolution of High-Throughput Experimentation in Pharmaceutical Development and Perspectives on the Future


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ABSTRACT: High-throughput experimentation (HTE) has revolutionized the pharmaceutical industry, most notably allowing for rapid screening of compound libraries against therapeutic targets. The past decade has also witnessed the extension of HTE principles toward the realm of small-molecule process chemistry. Today, most major pharmaceutical companies have created dedicated HTE groups within their process development teams, invested in automation technology to accelerate screening, or both. The industry’s commitment to accelerating process development has led to rapid innovations in the HTE space. This review will deliver an overview of the latest best practices currently taking place within our teams in process chemistry by sharing frequently studied transformations, our perspective for the next several years in the field, and manual and automated tools to enable experimentation. A series of case studies are presented to exemplify state-of-the-art workflows developed within our laboratories.

KEYWORDS: automation, catalysis, high-throughput experimentation, screening

1. INTRODUCTION

As pharmaceutical companies come under more pressure to accelerate the timelines of drug discovery and development, process chemistry departments must contribute to meeting this demand by delivering robust and efficient manufacturing routes to active pharmaceutical ingredients (APIs) faster than ever. This pressure extends to the academic space through an increased demand for novel chemical transformations.

In response to these challenges, new approaches to reaction discovery and optimization that allow for faster experimentation without sacrificing the quality of the results have been established and developed. One of these new approaches to process chemistry is high-throughput experimentation (HTE).1,2 Inspired by many techniques first developed in the biology space, chemistry has adapted and advanced the use of HTE as a means to keep up with the increasing demand of pharmaceutical timelines and an ever-expanding toolkit of chemical reactions.

HTE can broadly be defined as the workflow of running multiple reactions in parallel. These sets of reactions are rationally designed to answer specific chemical questions (e.g., probing reaction mechanisms or examining the scope of reagents for desired chemical transformations) and to achieve
specific process chemistry goals (such as determining the ranges of reaction parameters which still give acceptable quality products). The overarching goal can be achieved practically in the laboratory in many different ways, utilizing varied equipment and consumables, with or without the aid of laboratory automation. As such, laboratories which routinely use HTE have developed tailored approaches and workflows to best enable their organizational needs.

In the past decade, HTE has seen a sharp increase in adoption in major pharmaceutical companies, and currently most of these companies have dedicated groups of technical experts to run and maintain an HTE platform. HTE has also seen adoption in academic institutions such as the University of Pennsylvania, which allows students to use this technology for their research projects. HTE is now used in many companies not only to accelerate the optimization of a route (including reaction conditions, work up, and isolation) but also to ensure wider coverage of chemical space than possible with one-factor-at-a-time optimization. More recently, significant advances in laboratory automation and analytical technology have enabled HTE workflows to become even more efficient, utilizing less material to run more experiments with less human intervention required.

In this review article, we aim to provide a comprehensive view of how HTE is being used in pharmaceutical companies for chemical transformations. The perspective is focused on chemical transformations which are intended for scale-up, and the primary objective is data generation and not necessarily chemical transformations which are intended for scale-up, and the stability/commercial availability of the boronic acid (or equivalents). As such, it is ideally suited to easily remove unwanted metal impurities to regulatory specifications (typically <20 ppm). Other common catalytic reactions include asymmetric hydrogenation, Pd-mediated borylation, and Ullmann coupling. As with heterogeneous catalysis, asymmetric hydrogenation relies on the availability of specialist HTE-hydrogenation equipment and bespoke ligand libraries. The Ullmann reaction has seen divergent uptake among pharmaceutical companies and is the most common coupling reaction within AZ. This may reflect the perceived benefits of Pd-mediated versus Cu-mediated couplings within the companies. AZ’s perspective is that Cu-mediated chemistry is greener, uses cheap and patent-free ligands, and is easier at removing the current data relates to small molecule synthesis (typically <600 molecular weight), and HTE screening outside this environment is not within the scope of this review.

Regarding the reactions studied by HTE groups, we collated our survey responses and listed reaction type data as a percentage per institution to normalize the results (Table 1). Data from Princeton is not included because they currently only perform photomediated catalysis. The reaction types are sorted from largest to smallest by their industry-wide average. Importantly, this data is not an analysis of total screens run but rather of total projects being investigated by HTE teams.

Biocatalysis is almost uniformly the most common reaction type screened in the pharmaceutical environment. We have not further broken down the biocatalysis classification as was done with chemo-catalytic reactions, since the focus of the current perspective is on chemical transformations. We do however note that the most popular biocatalysis transformations include transaminases, keto-reductases, and hydrolases. For chemical transformations, Suzuki–Miyaura reactions are usually the most common of the cross-coupling reactions, followed by Buchwald–Hartwig amination. The prevalence of Suzuki–Miyaura coupling is tied to the broad substrate scope of the reaction, tolerance to acidic protons, mild conditions, and the stability/commercial availability of the boronic acid (or equivalents). As such, it is ideally suited to easily find the optimum conditions via HTE and deliver robust process control in API manufacture. Only at Merck are Suzuki–Miyaura couplings less prevalent in the HTE group, but this is because the reaction is often screened earlier in medicinal chemistry or provides sufficient yield without HTE intervention. Similarly, Buchwald–Hartwig aminations have a broad substrate scope and readily available amine starting materials. Heterogeneous catalysis is the fifth most common reaction type and typically includes protecting group removal (e.g., Bn, Cbz), reduction of double and triple bonds, and nitro group reductions. These reactions require specialist equipment for handling high pressure chemistry and may explain the differing data between companies.

Among noncatalytic reactions, chiral salt resolution, solvent/base, and scavenger screening were the most common. Scavenger screening’s prevalence highlights the empirical nature of removing unwanted metal impurities to regulatory specifications (typically <20 ppm). Other common catalytic reactions include asymmetric hydrogenation, Pd-mediated borylation, and Ullmann coupling. As with heterogeneous catalysis, asymmetric hydrogenation relies on the availability of specialist HTE-hydrogenation equipment and bespoke ligand libraries. The Ullmann reaction has seen divergent uptake among pharmaceutical companies and is the most common coupling reaction within AZ. This may reflect the perceived benefits of Pd-mediated versus Cu-mediated couplings within the companies. AZ’s perspective is that Cu-mediated chemistry is greener, uses cheap and patent-free ligands, and is easier at removing the

### Table 1. Survey Questions

<table>
<thead>
<tr>
<th>entry</th>
<th>question</th>
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<tr>
<td>1</td>
<td>What reaction types are being studied in your group?</td>
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<tr>
<td>2</td>
<td>What reaction types will be studied in your group in 3–5 years?</td>
</tr>
<tr>
<td>3</td>
<td>What is the stage of pharmaceutical development of your HTE projects?</td>
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<td>4</td>
<td>Are HTE projects carried out by specialists or lab chemists?</td>
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<td>5</td>
<td>What ratio of projects in your group study nonprecious vs precious metals?</td>
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2. **HTE METRICS**

In this section, we provide the reader with a context for the breadth of reaction discovery and optimization that may be accomplished via HTE and provide insight into the most common reactions conducted within pharmaceutical and academic laboratories. A survey was sent to seven chemistry HTE groups—Amgen, AstraZeneca (AZ), Bristol-Myers Squibb (BMS), GlaxoSmithKline (GSK), Lilly, Merck, and Pfizer—and a survey subsection to HTE academic laboratories situated at Princeton and University of Pennsylvania (UPenn, Table 1). Pharmaceutical data is provided by process chemistry groups except for Lilly, whose team works at the medicinal and process chemistry boundary. We queried these groups to analyze data from 2013 to 2017 (Merck data is 2015–2017) and identify the reaction types being evaluated for each HTE project. The
metal contaminants to the required specification, making this a prioritized method for C−O and C−N bond formation. Our data includes a surprising number of low-ranking transformations as well. The lack of Pd-mediated C−H activation reactions is notable despite a large volume of academic literature on the subject. Process chemistry challenges may include high reaction temperatures, high metal loading, selectivity, substrate scope, or the need for directing groups.

<table>
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<th>Reaction type</th>
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<th>Amgen</th>
<th>AZ</th>
<th>BMS</th>
<th>GSK</th>
<th>Merck</th>
<th>Pfizer</th>
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Another notable data point is the small value for non-Suzuki–Miyaura C–C cross-coupling reactions. As stated above, this probably relates to a combination of stability of organometallic nucleophiles, ease of screening, functional group tolerance, and the robustness of the Suzuki–Miyaura coupling. Catalytic amidation reactions were also widely overlooked among HTE groups, despite its potential to minimize process mass intensity (PMI), improve the environmental burden, and lower costs of a process. Despite some recent key advances, the need for copious amounts of molecular sieves as drying agents and/or high temperatures has so far limited this reaction type’s utility. A step change to a lower temperature catalytic amide formation
with a wide substrate scope without excess drying agents could shift the transformation toward wider applicability within an industrial setting.

As expected, the reaction types screened in an academic setting reflected the interests of the parent institution but maintained some alignment with industry results. Suzuki−Miyaura and Buchwald−Hartwig couplings were key transformations, as was photomediated catalysis, a field garnering lots of excitement among academic researchers. Little biocatalysis and no chiral salt screens were undertaken, indicating the university's current chemo-catalytic focus and preference for stereoselective reactions, respectively.

HTE teams were also asked to predict which transformations would be screened most often in 3−5 years (Figure 1). Surprisingly, little change was predicted from where things are today. The key reactions of biocatalysis, Suzuki−Miyaura coupling, Buchwald−Hartwig amination, Pd-borylation, and asymmetric hydrogenation are still predicted to be workhorse transformations. Key changes appear to be increased adoption of photoredox catalysis, Pd-mediated C=H activation, and catalytic amide formation. While HTE techniques have seen rapid innovation over the last several years, these changes have yet to be reflected in the chemical transformation to be studied by HTE for process development chemistry. As a note to the academic community developing novel methodologies, adhering to governing principles such as the SELECT criteria will support greater deployment in an industrial setting.

Our survey found that process chemistry HTE groups favor early engagement in order to best influence route selection, despite the high attrition rate of assets in drug development (Figure 2). This approach can minimize costs through reduced plant time, improved flexibility and speed of supply of drug substance, and lowered material requirements. An early change also has less regulatory burden and avoids expensive bridging toxicological studies to support an altered impurity profile. In contrast, post approval changes can be very expensive to implement, with large regulatory hurdles making route changes after regulated starting materials (RSMs) unlikely in all but the most financially compelling products. Despite these barriers, the opportunities to change prior to RSMs in launched products remains convincing, as cost savings can be attributed directly to lowered cost of goods over the product’s market lifetime. While these changes can be organizationally more challenging (different geographical locations or management structure), the opportunity exists for improving adoption of HTE for new or amended routes in this area.

Within the pharmaceutical environment, the majority of HTE work is carried out by specialist teams who confirm that experimental work is carried out accurately and insightfully to ensure that project teams have quality data to improve their processes (Figure 3). While this has historically been the case,
there is clear motivation to adapt to a more user-led approach. This may be motivated by reduced reliance on complicated deck-based robot systems that require substantial training to maintain and operate. While deck-based systems can be best used for standardized platform screens, such as solubility or salt-formation studies, more modular systems for automated weighing, solvent evaporation, liquid dispensing, reaction agitation, and sampling have a lower training burden and enable all chemists to utilize HTE. Academic institutions like UPenn and Princeton are ahead of industry groups in this regard, with end-users performing the vast majority of HTE screens. We expect this paradigm to progress into process development in the coming years.

Key catalytic reactions have usually relied on expensive, scarce metal catalysts such as Pd, Pt, Ir, and Rh. These rare elements present a substantial geopolitical risk to ongoing supplies due to the limited number of countries that supply the market. As such, there has been a push to carry out similar and new chemical pathways with more earth-abundant metals.10 We have found signs that nonprecious metal use is significant (Figure 4), but additional attention is needed within process development and academic settings for the discovery of novel couplings and asymmetric hydrogenations with abundant metals and cheap, available ligands.

3. HTE EQUIPMENT

A brief survey of industrial and academic HTE laboratories was conducted to review equipment used frequently within the HTE ecosystem (Table 3). In order to successfully enable HTE technology within the laboratory, a core set of nonautomated, or “manual”, equipment is usually required to allow the end user to quickly design, execute, and analyze HTE projects. Within more specialized workflows and groups, laboratory automation begins to play a greater role.

HTE adoption relies on the ease of use of tools to dose/sample reagents and substrates at the micromole scale. Single and multichannel pipettors (Eppendorf, Rainin, Fisher, VWR) are universally found in the laboratories surveyed, reflecting their low barrier to entry and convenience, alongside an array of 24-, 48-, and 96-well plates of varying volumes (1, 2, and 4 mL; Unchained/Freeslate, Analytical Sales & Services, ChemGlass, and custom internal design). Most laboratories utilize nitrogen filled gloveboxes (mBraun, Vacuum Atmospheres) for the majority of screen setup, and most groups surveyed have nitrogen purge boxes for compound collection storage and other nonpristine inert atmosphere critical workflows. Centrifugal evaporators (Genevac HT-X, EZ-2, Thermo-Fisher) located within a nitrogen filled glovebox are used for solvent removal. Low-barrier HTE approaches use nitrogen blow-down tools (Analytical Sales & Services) for solvent removal. Reaction agitation/heating is achieved with tumble stirrers (V&K Scientific, Unchained/Freeslate Core Module), hot plate stirrers (IKA), incubators (ECHO Therm), heater/cooler shakers (Eppendorf Thermomixer C), and in some cases custom designed shaker/heaters. Reactive gas delivery platforms, useful for hydrogenations and carbonylations, are prevalent in most groups and allow experimentation at atmospheric and elevated pressures. These systems offer singleton reaction screening (Biotage Endeavor) and up to 96-well-plate screens (Unchained/Freeslate, Swagelok).

HTE can be augmented by automated platforms, such as liquid and solid handlers, to enable accurate and efficient screen setup. Consequently, some form of automation is employed by all groups surveyed. The overall usage of automated tools is lower compared to the nonautomated “manual” tools, possibly reflecting nontrivial setup and complexity of these systems; nevertheless, Unchained Laboratories/Freeslate/Symyx systems (core module 1–3, Junior) are listed across most teams surveyed. These systems offer either standalone liquid handling or liquid-solid handler capabilities, although most groups opt for separate liquid handling and solid handling platforms. Other vendors such as Chemspeed Swing, Swing XL, Tecan Freedom EVO, Thermo Matrix 2 × 3, Andrew Alliance, and Rainin Benchsmart are also utilized for liquid handling. Manually weighing solid reagents presents a challenge, especially when one considers multiple charges of the same or different reagents at milligram quantities! To address this, groups utilize solid handling platforms from a mix of vendors: Mettler-Toledo (Quantos QS5 and QX96, and XPE20S), Unchained Laboratories (Solid-Junior), Chemspeed (SDU, GDU-P FD), and JKen (Eclipse).

Approximately 80% of automated platforms are situated under an inert nitrogen atmosphere, with the remaining 20% located on the bench, presumably being used for non-air/water sensitive applications.

Analysis of reaction outcome becomes the major bottleneck of processing HTE screens. Prior to the development of ultra-high-performance liquid chromatographic systems (UPLC), one could expect to spend 16–18 h of analysis time for a single 96-well plate12 in order to collect high-quality data. UPLC systems provide rapid analytics allowing for superfast method development (20–30 s in some cases) while maintaining high-quality data. All groups utilize UPLC systems to some extent (Agilent 1290, Waters Classic, H-Class, I-Class, and Shimadzu). Mass spectrometers are coupled to approximately 40% of these systems. High-performance liquid chromatography (HPLC) is still in use across the groups surveyed but with lower usage when compared to UPLC systems. Finally, supercritical fluidic chromatographic systems (SFC) are being adopted, with low to medium usage reported (Waters UPC2, Agilent Aurora/1260). Gas chromatography with mass detector (GC-MSD) has limited use.

HTE can have its greatest impact when manual, automation, and analysis tools become rationally integrated. The ensuing case studies will provide greater detail for how different HTE teams in industry can convert their research tools into streamlined workflows.

4. HTE CASE STUDIES

4.1. Case Study #1: Application of High-Throughput Experimentation to a Suzuki–Miyaura Step for the Synthesis of a PCSK9 Inhibitor. Compound 1 (Figure 5) is a small molecule inhibitor of proprotein convertase subtilisin/kexin type 9 (PCSK9) developed at Pfizer.12 PCSK9 is a protein involved in the maintenance of plasma low density lipoprotein...
cholesterol (LDLC), and its inhibition can lead to low levels of 
LDLC, often called "bad cholesterol", and decrease the risk of 
coronary heart disease. This case study describes our HTE 
efforts to install the tetrazole prodrug moiety of the molecule via 
a Pd-catalyzed Suzuki−Miyaura cross-coupling.

Transition-metal-catalyzed chemistries are carried out at 
Pfizer inside a glovebox with an oxygen level below 10 ppm. The 
glovebox houses an Unchained Laboratories CM3 platform that 
is employed for liquid handling and controlled by Laboratory 
Execution and Automation (LEA) software. A very large 
percentage of our screening work involves transition-metal-
catalyzed (Pd, Ni, Cu, Fe) cross-coupling reactions to support 
process chemistry projects, and over the past few years, our 
group has designed highly efficient HTE workflows that allow us 
to provide quick turnaround to meet our process chemistry 
colleagues’ timelines.

Screens are carried out in 96-well plates containing 1 mL glass 
vials, to which parylene-coated stir bars are added using a 96-stir 
bar dispenser; the vials are placed in a 96-well aluminum block, 
and after reagent addition, the plates are sealed using a solvent-
resistant PTFE mat, followed by two silicon rubber mats, and 
then secured with a stainless steel cap that is screwed to the 
aluminum block using seven screws; the plate is then stirred 
using a tumble stirrer (typically at 300−500 rpm) and heated at 
the desired temperature on the CM3 deck inside the glovebox.

For a typical Pd-catalyzed Suzuki–Miyaura cross-coupling, 
our group has developed several types of plates for 96-well 
screening. The choice of plate depends on the stage and 
timelines of the project:

1. Plate containing 6 Pd(0) and 18 Pd(II) precomplexed 
and commercially available Pd sources (4 sets of 24 Pd 
sources per plate): this plate is generally employed for fast 
moving, early stage projects, for which cost is not an issue. 
It has broad applicability in cross-coupling and allows for 
the screening of, e.g., two solvents and two bases per plate. 
This plate was designed with a fit-for-purpose approach in 
mind.

2. Plates containing achiral ligands: We have developed two 
plates each containing 48 ligands (2 sets of ligands per 
plate) as well as a plate containing 96 less commonly used 
ligands, for a total of 192 ligands, mostly mono- 
and bidentate phosphines. In combination with a suitable Pd 
source, a very broad ligand screen can be carried out in a 
short time. This approach is usually followed for advanced 
and late stage projects heading toward commercialization, 
for which cost becomes one of the limiting factors.

3. Plate containing 32 Buchwald palladacycles (third and 
fourth generation) and 12 Johnson Matthey precom-
plexed allyl/crotyl palladium sources (all 44 commercially 
available): this plate is employed for particularly difficult 
cross-couplings, in which a very active Pd catalyst 
is required. These Pd sources feature facile generation of 
the active Pd species and display applicability across a 
wide range of cross-coupling types. Drawbacks are a 
higher cost, intellectual property issues due to patented 
technologies, and the possibility that obtaining the Pd 
source in bulk can be problematic.

A key step in our process is exploiting the liquid handling 
capabilities of the Unchained Laboratories CM3 platform for the 
generation of 96-well plates containing Pd sources and ligands. 
Metal sources and ligands are dispensed as solutions/slurries in 
THF (50−100 μL/well) to achieve 1 μmol of metal/well 
(equivalent to 5 mol % based on limiting reagent). The solvent is 
then allowed to evaporate inside the glovebox, and the plates are 
capped and stored in a drybox under nitrogen at ambient 
temperature until the day of the screen.

On the day of the screen, the following steps are implemented:
1. The plate is transferred back into the glovebox from the 
drybox.
2. If a plate containing preplated ligands is employed, the 
metal source is dispensed to the wells as a stock solution in 
THF (50−100 mL) with the CM3 platform or via pipet 
and the metal/ligand mixture in THF is vortexed or 
stirred uncapped to precomplex the ligand to the metal 
source (30−60 min) and evaporate the solvent. Traces of 
THF can then be removed under a vacuum inside the 
glovebox using an evaporator.

Scheme 1. Medicinal Chemistry Conditions for Suzuki−Miyaura Cross-Coupling
3. If a solid base is required (2–3 equiv), the base is dispensed at this stage in an automated fashion with Mettler-Toledo’s QX96 platform, which is housed in a purge box. Alternatively, solid dispensing can be manually accomplished using a Biodot pipet for solids if a higher degree of tolerance in the dispensed mass is acceptable. If an aqueous or liquid base is employed instead, see step 5 below.

4. A mixture of both reactants (typically 10–20 μmol of limiting reagent) and internal standard (if required) in the desired solvent is added manually via pipet to each well.

5. Aqueous base (2–3 equiv) is added manually via pipet. If a liquid organic base is required, it can be added neat at this point via pipet or combined with the reactants/internal standard in step 4.

6. The plate is then capped and stirred at temperature inside the glovebox on the CM3’s deck. After the desired reaction time, each well is diluted with an appropriate solvent that quenches the reaction and brings all components into solution. If solids still remain, the plate is centrifuged to allow them to settle. The plate is analyzed by UPLC-MS if a chromatophore is present or by GC-MS. The analytical data is then processed using Waters Empower software, exported to Excel, and visualized using TIBCO Spotfire software.

Regarding the preparation of 1, our Medicinal Chemistry group developed conditions that employed Pd(OAc)₂ and CataXium A to cross-couple boronic ester 3, obtained via borylation of aryl bromide 2, with 4-iodopyrazole 4 to afford Suzuki–Miyaura product 5 (Scheme 1) as the penultimate intermediate en route to API 1.

However, the isolation of 5 required chromatographic purification to remove phenol 6, homocoupling dimer 7, and prodrug cleavage byproduct 8 byproducts, which afforded a low-yielding process (Figure 6). We hypothesized that the formation of 6 and even though other alternatives such as Negishi cross-coupling were investigated, the unsatisfactory results from these studies led us to focus our attention on the optimization of the Suzuki–Miyaura cross-coupling to turn it into a scalable process to generate kg quantities of API.

PCSK9 was a fast-moving project due to tight timelines to generate the first clinical batch. As a result, our group decided to initially investigate 24 commercially available precomplexed Pd(0) and Pd(II) sources plated out in 96-well format to couple pinacol ester 3 with iodopyrazole 4. A critical discovery by our Medicinal Chemistry group was the need to employ a very mild base in this coupling to avoid significant levels of prodrug cleavage byproduct 8. CsF was identified as the preferred base to facilitate the Suzuki–Miyaura coupling while minimizing carbonate prodrug cleavage to the unprotected tetrazole, as has been previously disclosed in a Pfizer publication on a related compound in this series.

The reactions were set up on a 5 mg (8.9 μmol) scale of boronic ester (limiting reagent) and 1.1 equiv of iodopyrazole 4 per well. Three solvents were tested (dioxane, toluene, and 2-MeTHF) in a 9:1 organic solvent/water ratio at 85 °C over 18 h using 4,4′-di-tert-butyl biphenyl as internal standard. UPLC analysis identified PdCl₂(DCyPF), PdCl₂(dpff), Pd(PCy₃)₂, and dichloro[bis(dicyclohexylphosphinophenyl) ether]-palladium(II) as the best catalysts in terms of Suzuki–Miyaura product/internal standard ratio (over 95% conversion based on boronic ester 3 consumption) while minimizing the formation of dimer 7 and prodrug cleavage byproduct 8. Phenol 6 was never detected, as it presumably forms in the presence of oxygen and our screen was carried out inside a glovebox with <10 ppm of O₂. Toluene proved superior to dioxane and 2-MeTHF in terms of Suzuki–Miyaura product/internal standard ratio.

On the basis of these results, a second screen was carried out that focused on these four precatalysts and tested a broad range of solvents (toluene, THF, 1,2-dimethoxyethane (DME), MeCN, t-AmOH, DMF, DMAc, NMP, and iso-propyl acetate (IPA)c): 9:1 organic/aqueous), with CsF as base, at 70 °C (THF, MeCN) or 85 °C (rest of solvents). Results from this screen showed PdCl₂(DCyPF) in toluene and Pd(PCy₃)₂ in toluene, DME, t-AmOH, and IPA to be clearly superior, providing complete conversions with less than 5% prodrug cleavage and trace or no homocoupling product. NaHCO₃ was investigated as an alternative mild base that would eliminate the need to avoid glass-lined tanks in our kilo lab facility, but it was outperformed by CsF for both Pd sources.

Despite their similar performance, we chose to focus on PdCl₂(DCyPF) over Pd(PCy₃)₂ because Pd(II) sources are more air-stable and easily handled in a manufacturing facility than Pd(0) sources. Thus, further screening tested Pd loading (1, 3, and 5 mol %) as well as temperature (70, 80, and 90 °C) in aqueous toluene. Samples were manually pulled at 1, 4, 6, and 24 h to determine the kinetic profile as well as impurity levels over time (Figure 7). Only reactions at 3 and 5 mol % Pd showed full consumption of boronic ester (blue line). The amount of Suzuki–Miyaura product (yellow line) peaked at around 4 h in most reactions and then started to decrease as increasing amounts of prodrug cleavage byproduct 8 (red line) began to be generated. This decomposition was favored at higher reaction temperatures (90 °C). No homocoupling byproduct was detected in any of the reactions (green line).

As a compromise between conversion, Pd loading, and prodrug cleavage byproduct level, we selected 3 mol % Pd at 70 °C for further development, as these conditions afforded full...
conversion to product in 4 h or less while maintaining the amount of prodrug cleavage below 3%. To test the scalability of this process, a 2 g scale reaction was carried out in an Easymax 50 mL reactor with mechanical stirring at 70 °C in toluene. Surprisingly, very low conversion was observed (30−40%); increasing the reaction temperature to 80 or 90 °C or charging additional Pd precatalyst had only minor effects. While the lack of scalability remains unclear, the biphasic nature of the mixture and switching from magnetic to mechanical stirring are possible issues.

In view of these results, we decided to investigate the use of Pd(PCy3)2 as an alternative. This precatalyst would avoid the Pd(II) to Pd(0) reduction step, ruling out a possible factor for the lack of reproducibility seen on larger scale. A broad solvent screen was carried out that included THF, 2-MeTHF, 1,4-dioxane, IPA, and t-AmOH at 70 °C (THF, 2-MeTHF) and 90 °C (1,4-dioxane, IPA, t-AmOH) (9:1 organic/water ratio; 20 mL/g limiting reagent) with Pd(PCy3)2 (5 mol %) and aqueous CsF as base (3 equiv). Reaction sampling was carried out at 1, 2, 4, 6, and 18 h. Both 1,4-dioxane and IPA afforded ≥98% consumption of boronic ester (limiting reagent; 1.1 equiv of aryl iodide) to product in 2 h, and most importantly, no prodrug cleavage or dimer formation byproducts were detected. When these two conditions were scaled up in an Easymax reactor on 1 g scale, slower reaction rates were observed in both cases: 5 h to completion in dioxane and 20 h to completion in IPA. Even though no prodrug cleavage was observed, dimer formation was at 2.7 and 6.1% in dioxane and IPA, respectively. In addition, reactions carried out on an even larger scale tended to produce increasing amounts of dimer. Further investigation determined that the cause of dimer formation was adventitious oxygen. We found that a reaction set up inside the glovebox and subsequently briefly exposed to air outside the glovebox afforded higher levels of dimer compared to an air-free control. However, no phenol byproduct was observed in the reaction exposed to air or in the previous scale-up experiments, which seem to indicate that either phenol formation is due to a factor other than adventitious oxygen or that Pd(PCy3)2 minimizes its formation. The true cause for the absence of phenol byproduct still remains unidentified at present. Under optimized conditions, reactions were sparged subsurface with nitrogen gas prior to the addition of the Pd catalyst and routinely afforded desired product in 92−94% isolated yield with no detectable amounts of dimer or phenol. As an additional precaution, Pd(PCy3)2 was weighed out inside a glovebox on the same day that the reaction was run, as samples of catalyst that had been kept in a sealed container outside of the glovebox for several days turned from white to pale green even under refrigeration, which was an indicator of exposure to oxygen.

Despite not being a desirable solvent in a manufacturing setting for toxicity reasons, dioxane was finally selected for the preparation of early batches for clinical studies due to its superior performance. A decision was made to investigate greener alternatives at a later stage of development.

With these precautions in place, the Suzuki−Miyaura cross-coupling step was successfully scaled up in our kilo lab facility to produce multi-kg quantities of intermediate 5. Further details on the optimization that led to the final Suzuki−Miyaura coupling conditions used on scale will be described in a future publication. The power of combining automation and HTS has been demonstrated through the discovery of an efficient catalyst for a challenging Suzuki−Miyaura cross-coupling that allowed us to...
In its synthesis, the conversion of aniline to hydrazine through diazotation and tin reduction could effectively reduce the aryldiazonium to the desired product in 75% yield. However, the removal of the superstoichiometric tin salts from the reaction stream and the use of tin for the preparation of a pharmaceutical intermediate presented significant obstacles on a large scale. Thus, a greener option utilizing sodium sulfite was identified, although highly variable yields were observed for large scale (>20 kg) batches (70–86%). To highlight the severity of the latter case, a hypothetical 1 t synthesis of 10 would require 1092 kg of 9 if the yield were 86% and an input of 1342 kg if the yield were 70%, a difference of 250 kg! It was envisioned that a comprehensive HTE effort could identify reaction conditions with improved robustness and greenness.

Prior to initiating our HTE studies, it was important to align our experimental design with the goals of the project by balancing existing in-house knowledge of the transformation with information available in the literature. This step was necessary to avoid lead conditions that would present new downstream problems requiring subsequent rounds of HTE. With this perspective in mind, it was envisioned that the study would focus on the reduction of aryldiazonium chloride 12, derived from NaNO2 and HCl (Scheme 3), as opposed to alternative aryldiazonium salts prepared through different means. Aryldiazonium 12 had already been safely prepared multiple times on a >50 kg scale, and this system presented a homogeneous slurry that could be exploited for the execution of the HTE study.

Having established 12 as the reactive intermediate, the other variables for consideration were the reductants and solvents. As inorganic sulfur-based reducing agents had already been demonstrated as viable on a multi-kilo scale, and likely offered the greenest path forward, it was envisioned that a large portion of the study would focus on this general class of reductants. A design was formulated that balanced precedence while inviting opportunity for serendipity through the incorporation of a targeted selection of organic, phosphorus, and metallic reductants. As the diazonium salt was prepared in water, the ideal path forward would be an entirely aqueous system. Again, to achieve a balanced design, additional solvents would be added as so as not to confine the study too narrowly.

The finalized experimental design contained a total of 96 experiments where sulfite-based reagents comprised 66% of the plate with six total reducing agents at varied equivalents, five solvents, a sparse array with sodium hydroxide as an additive, and varied concentrations. The remainder of the design incorporated seven different reducing agents at set equivalents with four solvents at a set concentration (Table 4). This strategy...
would more deeply interrogate the promising sulfitel-based conditions while also providing an adequate assessment of a diverse pool of reducing agents.

Table 4. Class Variables for the HTE Study

<table>
<thead>
<tr>
<th>sulfitel reductants</th>
<th>solvents (sulfitel)</th>
<th>prospective reductants</th>
<th>solvents (prospective)</th>
</tr>
</thead>
<tbody>
<tr>
<td>potassium metabisulfitel</td>
<td>AcOH</td>
<td>ascorbic acid</td>
<td>AcOH</td>
</tr>
<tr>
<td>sodium bisulfitel</td>
<td>DMAc</td>
<td>dithiothreitol (DTT)</td>
<td>MeCN</td>
</tr>
<tr>
<td>sodium dithionite</td>
<td>MeCN</td>
<td>iron(II) chloride</td>
<td>THF</td>
</tr>
<tr>
<td>sodium metabisulfitel</td>
<td>THF</td>
<td>oxalic acid</td>
<td>water</td>
</tr>
<tr>
<td>sodium sulfite</td>
<td>water</td>
<td>sodium formate</td>
<td></td>
</tr>
<tr>
<td>sodium thiosulfate</td>
<td>tris(2-carboxyethyl)phosphate (TCEP)</td>
<td></td>
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<tr>
<td></td>
<td>PCy₃</td>
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<td></td>
<td>PPh₃</td>
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</table>

To execute the HTE, reducing agents were dispensed to a 96-well plate via an automated powder handling protocol (Unchained Laboratories CM3). The solvent and 5 Naq NaOH were added to the desired wells on the benchtop, and the plate was equilibrated to 0–5 °C over 20 min. Concurrently, a calculated 14.2 wt % stock slurry of 12 was prepared according to the existing procedure and was dispensed to each well as a homogeneous slurry by electronic pipettor (BrandTech Handy-Step); additional solvent was added to wells designated for lower concentration. The plate was transferred to a 0–5 °C CM3 stirring deck, flushed with nitrogen using a custom-made plate housing connected to house nitrogen, sealed, and stirred at 300 rpm for 2 h followed by sampling for UPLC-MS analysis.

The analysis of the plate was split into two groups on the basis of the structure of the design, the sulfitel reagent group, and the prospective reagent group. The prospective reagent group showed that dithiothreitol (DTT) and tris(2-carboxyethyl)-phosphine (TCEP) afforded modest amounts of product in certain cases and may provide the opportunity for further investigation and optimization (Figure 8). The other organic reductants, phosphines, and FeCl₂ reactions were largely ineffective.

The sulfitel reagent group was analyzed next (Figure 9). It was observed that water was the optimal solvent in most cases. Inspection of the median values for each reagent, as indicated by the horizontal line within the box for each respective reagent, revealed that sodium bisulfitel gave the highest area percents (APs). Furthermore, every reaction with sodium bisulfitel conducted in water afforded greater than 70 AP, regardless of the concentration of the reaction or the equivalents of the reducing agent across the range explored in this study. This was in contrast to other reagents that tended to show decreased AP of 10 with higher reducing agent equivalents.

The hits from the HTE were then subjected to more rigorous analysis and optimization. It was revealed that the robustness observed using the sodium bisulfitel in the HTE study was related to its increased buffering capacity during the reaction that maintained a slightly acidic pH (2–4). In contrast, the more basic sulfitel reducing agents that offered a much narrower window resulted in larger deviations due to small changes in reaction concentration or reagent equivalents. Under these conditions, it was found that deleterious SNAr side products were suppressed, resulting in higher yields. After continued optimization by the project team, the lead reaction conditions were successfully scaled to >100 kg and provided a yield of 85% across multiple batches.

In summary, the strategic application of HTE through thoughtful experimental design delivered an innovative solution to a unique challenge of aryldiazonium reduction. The HTE study enabled the use of existing intermediates and process knowledge, allowing for rapid optimization and technical transfer to an external vendor by the project team, while also

Figure 8. HTE results for prospective reducing agents. Visualization was prepared in RStudio.
providing confidence that the reagent pool had been thoroughly examined.

4.3. Case Study #3: Standardizing High-Throughput Screening of Heterogeneous Catalysts at GSK: A Case Study in Chemoselective Hydrogenation. One of the most frequently screened organic transformations at GlaxoSmithK-line is hydrogenation using heterogeneous catalysts, specifically the reduction of nitroarenes to anilines. Because of the molecular complexity of pharmaceutical intermediates and targets, a given substrate often has many potential sites of reduction; identification of active and chemoselective reaction conditions, including catalyst identity, solvent, and pH, is therefore paramount. To facilitate these efforts, the High Throughput Chemistry and Chemical Catalysis groups within GSK’s API Chemistry department have designed and validated a series of standardized high-throughput screens for hydrogenations; we also maintain a sizable library of commercial heterogeneous catalysts for more in-depth screening. Herein, we describe the hydrogenation of 2-bromo-4-chloro-nitrobenzene using our standard nitroarene hydrogenation screens. By using these preplated kits, a wide array of commercial catalysts can be rapidly evaluated, leading to identification of promising chemoselective catalyst candidates for further process development.

A particularly powerful application of HTE in catalysis screening is to simultaneously evaluate a large suite of heterogeneous catalysts. Unlike homogeneous catalyst systems, where skilled practitioners can select appropriate metal/ligand combinations using mechanistic rationales, choosing the right hydrogenation catalyst is not as straightforward. Many organic chemists are unaware of the wide range of reactivities exhibited by “5% Pd/C” and “5% Pt/C” catalysts. While reactivity guides for commercial catalysts are available from vendors, there are many hidden factors in the design and formulation of these systems that dramatically change their reactivity.

Chemoselective reduction of nitroarenes that also contain aryl halides is often carried out using a mixture of metallic iron and acid, such as Fe/AcOH or Fe/NH₄Cl, and a protic solvent. These conditions often provide chemoselectivity but are not desirable to use on-scale due to exothermicity, mixing difficulties, and problematic filtrations. Catalytic hydrogenation is a far more efficient alternative; however, selection of the catalyst, solvent, additive, reaction temperature, and hydrogen pressure are all key factors in obtaining the desired chemoselectivity and reactivity of this transformation. At the outset of development, it can be difficult to find an appropriate system using iterative optimization. To accelerate this process, the following reaction parameters are varied in the GSK standard aryl nitro reduction screens:

1. Catalyst: Pd or Pt on carbon (Pd/C, Pt/C) catalysts can be used to promote the reduction of aryl and heteroaryl nitro compounds to the corresponding amine. These same catalysts can be chosen for aliphatic nitro compounds, but the reaction conditions tend to be more vigorous to overcome the inhibitory effect of the alkylamine product. Substrates containing halides typically utilize Pt/C as the catalyst to minimize dehalogenation. Wet Pd/C or Pt/C catalysts (~50 wt %) are used to increase the safety and reactivity of these catalysts.

2. Solvent: Many different reaction solvents can be used, so solvent choice is generally dictated by substrate solubility. Lower alcohols are frequently employed, as are aprotic solvents such as THF and EtOAc. A low polarity solvent such as cyclohexane may also be used. Aprotic solvents inhibit dehalogenation, and protic solvents tend to increase the reaction rate. The addition of water as a cosolvent can also have beneficial effects on reaction rate, provided the organic components remain in solution.

3. Reaction pH: Many hydrogenations, especially those involving polar bonds, are accelerated by acidic media. Reduction of the nitro group to the amine can be achieved under neutral conditions; however, it may be necessary to

Figure 9. HTE results for the targeted study of sulfite reducing agents. Visualization was prepared in RStudio.
utilize slightly acidic conditions to offset the inhibitory effect of the amine product, which is heightened in the case of aliphatic substrates. Acidic conditions can also help to inhibit dehalogenation.

4. Modifiers and promoters: Modifiers such as organic bases, sulfur compounds, H₃PO₂, MnO₂, or ZnX₂ can aid reaction selectivity. Using Pt/C catalysts doped with V, Fe, Cu, or Ru can increase the rate of reaction and decrease or prevent the accumulation of a hydroxylamine intermediate. V dopants specifically are known to effect disproportionation of the hydroxylamine intermediate to the aryl nitro starting material and the desired product.³³

To efficiently evaluate these variables, GSK has developed two 48-well plate hydrogenation screens for reduction of aryl nitro compounds:

1. Plate 1 contains 34 different Pd/C- and Pt/C-based catalysts. Three of these catalysts are repeated four times to allow different solvents and an acid modifier to be evaluated within a single screen. This plate can be used for aryl and heteroaryl nitro compounds lacking halides or hydrogen sensitive groups (e.g., nitriles, ketones/aldoxides, olefins, O- or N-Bn, Cbz). The plate can also be utilized to screen for conditions to mediate aliphatic nitro compound reductions.

2. Plate 2 contains 19 different Pt/C catalysts and is specifically designed for halogenated aryl and heteroaryl nitro compounds. Two of these catalysts are repeated four times to allow different solvents and an acid modifier to be evaluated in a single screen.

Chemists can consider executing two rounds of screening using one plate. Round 1 can be executed at 23°C for 4–18 h. Round 2 can be executed at an elevated temperature to see if this reaction parameter affects conversion. On the basis of a 5–7% (w/w) catalyst loading, chemists need about 1 g of substrate per plate.

All solid heterogeneous catalysts are predispensed into 2 mL HPLC vials (each already containing a stir bar) using the Mettler Toledo QX96 solid handling automation located in a ventilated enclosure. These plates are then sealed and stored in the laboratory at ambient temperature for use at any time. Chemists need only add their reagents as stock solutions in the reaction solvents (at desired reaction concentration), followed by any additives such as an acid modifier. All 48 HPLC vials are then sealed using crimp septum caps, which are pierced with a needle to ensure gas ingress. The metal block containing the reaction vials is transferred to an HEL Cat96 pressure reactor, which automatically controls temperature, gas pressure, and reaction time.

After the desired reaction time, all 48 vials are decapped and the reactions are diluted with 1 mL of the appropriate HPLC solvent using an eight-channel multichannel pipet. The vials are then covered with a sealing mat and centrifuged to pelletize the heterogeneous catalyst to allow for sampling of the homogeneous supernatant. Next, HPLC samples are taken using an eight-channel multichannel pipet and transferred to an analytical block, where the samples are further diluted to HPLC sample concentration. The entire plate is submitted to an HPLC or LCMS as a batch, and the analysis is run with an appropriate
method. "Hits" are determined by wt/wt assay against a known amount of internal standard, or by calibration curve if a product marker is available. Mass balance and levels of byproducts are carefully analyzed. Transfer of the lead conditions to a larger scale reactor is enacted as soon as possible, as heterogeneous reactions often exhibit mass-transfer-limited kinetics. Sufficient hydrogen volume (headspace) and efficient mixing are also key factors in a successful reaction.

Commercially available arene 13 was used as part of a validation project for the two plate designs. This case study (Scheme 4) illustrates that, in addition to the desired aniline, two frequently encountered byproducts arise from dehalogenation (15) and hydrazine dimer formation (16).

Performing the hydrogenation of 13 using nitro reduction plate 1 leads to a broad range of results (Figure 10). The Spotfire plot shows the area percent of 14 as a function of catalyst identity, revealing that few systems are capable of achieving >50% (by area) conversion to the desired aniline, despite the fact that full conversion of substrate is nearly always observed. Further data analysis illuminates several key aspects of this reaction. First, as can be seen with two catalysts (one Pd and one Pt), there is a dramatic solvent effect (highlighted by the nonblue data points), with aprotic solvents leading to much greater AP product. This is important to note, since the preferred solvent for most catalytic hydrogenation reactions is methanol. Second, Pt-based catalysts generally outperform their Pd and Pd/Pt counterparts, with five catalysts achieving >50% conversion. This screen also assessed the impact of added acetic acid (not shown in data), which was ambiguous in this case.
Finally, the data from plate 1 reveal that divergent mechanistic regimes appear to be operating: a plot displaying AP desbromo-aniline 15 (y-axis) versus AP hydrazine 16 (x-axis) reveals two orthogonal sets of reaction conditions (Figure 11). The set favoring debromination is comprised of Pd and Pd/Pt catalysts, while that favoring formation of the hydrazine byproduct contains Pt catalysts, as well as one Pd catalyst in aprotic solvents. In contrast to 15, the hydrazine “dimer” is actually an intermediate en route to aniline 14 in the condensation mechanistic pathway (Scheme 5).

On the basis of the results from plate 1, we hypothesized that nitro reduction plate 2, which contains mostly Pt/C catalysts, would also deliver a number of positive hits. Indeed, a screen of this second plate revealed that many systems are capable of high conversion to product, with only the sulfided-Pd catalysts (denoted by ◆) giving the desbromo-aniline (Figure 12). Note that promoters/inhibitors (such as Cu or V as promoters or S as an inhibitor) appear to have little effect on the case study reaction. The combination of both data sets thus provides a solid foundation to further develop a nitro reduction process through appropriate catalyst and solvent selection.

The preceding application of HTE to the discovery of effective heterogeneous catalysts demonstrates the vastly different reactivities and selectivities exhibited by these catalysts, despite their simple “5% Pd/C” or “5% Pt/C” names. The data from nitro reduction plate 1 demonstrates that two distinct sets of reaction conditions are operative, with Pd in protic solvents leading to dehalogenation, while Pt catalysts and Pd catalysts in aprotic solvents generate more of the diarylhydrazine intermediate. Application of plate 2, with a greater breadth of Pt/C catalysts, resulted in the identification of several systems capable of generating >90 AP product in a chemoselective manner. This high-throughput approach to comprehensive chemical space screening in heterogeneous catalysis has become a standard way of working for process development activities at GSK, due in no small part to the standardization of screening designs and ease with which any member of the department can execute and analyze a screen.

4.4. Case Study #4: Practical Asymmetric Fluorination Approach. Aminoalcohol 27 is an intermediate in the synthesis of the potent BACE1 inhibitor LY2886721 (24). Over the course of the program, we also sought to prepare analogue 25 containing a fluorine at the bridgehead position of the bicyclic aminothiazine (Scheme 6). We hypothesized that fluorinated aminoalcohol 26b could provide access to fluorinated bicyclic aminothiazine 25. Herein, we describe our reaction screening and optimization to develop a safe, enantioselective, and scalable route for the fluorination of the aldehyde precursor of 26b, prepared from the available key chiral amino alcohol intermediate 27.

Lilly’s Optimization and Parallel Synthesis Lab (OPSL) integrates commercially available laboratory automation with custom developed software and hardware to conduct high-throughput reaction optimization and automated parallel synthesis of compound sets. This approach allows our Automation Group to design, implement, and operate a system that effectively provides teams with data and materials. The OPSL system currently consists of seven automation platforms.
capable of performing independent laboratory processes, including reagent and reaction preparation, reaction vessel taring and weighing, solid and liquid dispensing, reaction incubation, automated workup, and reaction monitoring. These platforms are logically connected through a central database and custom-developed laboratory automation and information management system. This efficient design provides the automated laboratory tools with a large degree of flexibility, allowing our group to incorporate new laboratory automation technologies and easily develop additional automated synthesis and reaction optimization workflows (Figure 13).

Autolab, a scientific informatics software, was developed to manage data flow and communicate experimental protocols across the OPSL automation platforms. Relying on a central relational database implemented using Oracle, Autolab acts as the focal piece to enable connectivity between electronic notebooks (eLN), experimental designs generated with JMP (Design of Experiments software), automation execution and control software called Experiment Preparation Software (EPS), and the final reporting of automated data analysis and results back to the eLN. Autolab performs all of the calculations required for each reaction, including stock solution preparations and amounts to be dispensed (either as solids or liquids). Experimental procedure and operational steps are also defined: dispensing, inert atmosphere purge, stirring, heating rate, incubation, aliquoting, and quenching. EPS uses this protocol to command the instrument, generating the corresponding scripts required for automated experimentation. After reaction sampling and analytical data processing, reaction results, including raw data for individual reactions, can be directly accessed within Autolab. Conversion and substrate and product purities are calculated and shown in a 96-well format from the HPLC-MS analysis. Reaction screening analysis can be customized to focus on multiple parameters such as conversion, impurity profile, or final product purity.

In terms of hardware, instruments are located inside a chemical fume hood and are running bespoke software connected to the database containing all experiment information. Stock solution/suspension recipes are retrieved from the database and prepared using an in-house customized Tecan liquid handler. The instrument labels and weighs the tubes, adds the appropriate amount of solvent, and caps the tubes in a fully automated process. Most solids are dispensed using a customized Chemspeed Flex Powerdose with GDU P-fd containers directly in the required tubes. This technology is implemented in an automated Flex platform that incorporates SWILE dispensing technology when only a limited amount of solid reagent is available. Alternatively, for nonautomatable solid dispensing, manual weighing is an option.

Reactions are performed using 8-tip Tecan liquid handlers that have been adapted to integrate a 96-well format H+P Variomag96 reactor that can fit either 8 mm × 100 mm or 16 mm × 100 mm reaction tubes to hold 50 μL to 1 mL final volume reactions. Reaction tubes are provided with individual stirring bars. We have designed a lid for this reactor that allows sealing of all tubes at once, inert atmosphere purge of the tubes, and aliquoting without removing the lid. The reaction block is connected to a Huber cryostat that operates from −65 to 165 °C. A CEM microwave is also integrated to an automated crimper—decrimper system to explore additional reaction conditions. A second 48-position H+P Variomag48 block is integrated in order to fit the stock solutions/suspensions required for the screening. This block holds either 16 mm × 100 mm or 25 mm × 100 mm tubes with individual stirring bars and can be heated and purged with inert atmosphere. Twelve additional solvent bottles (100 mL) are stored on an IKA plate in order to adjust the final reaction concentration. A Tecan SPE block is included to take filtered reaction aliquots in 96-well microplates. These samples are analyzed by an Agilent 1100 LCMS instrument set at 214 nm. Electrospray ionization mass

Figure 13. OPSL reaction optimization workflow.
spectrometry (ESI-MS) measurements are performed on an MSD quadrupole mass spectrometer (Agilent Technologies) interfaced to the above HPLC system. MS measurements are acquired in positive ionization mode over the mass range of $100^{−700}$. Data acquisition and integration for LC/UV and MS detection are performed using Chemstation software (Agilent Technologies).

Workflows are designed according to experiment objective, project needs, timelines, factors to be studied, and scope. In a generic OPSL reaction optimization experiment, the following workflow is executed:

1. Reaction design: A user inputs reagents and variables to generate an eLN experiment that is exported as an XML file.

2. Experiment generation: The resulting XML file is imported into Autolab. The user specifies the phase of chemical reagents (solid, liquid, or stock solution with the corresponding concentration), so quantities can be calculated. Information within Autolab is stored in a central database that is shared by all lab instruments.

3. Design of Experiment (DoE): JMP software embedded within Autolab prepares an appropriate DoE and experimental plan. A procedure for distribution of reagents is automatically generated and can be exported to an Excel report file.

4. Platform operations: A drag and drop protocol in Autolab defines the sequence of operations for different modules (agitation, dispensing, purging, incubation) for experimental execution.

5. Experiment execution:
   a. Required solution recipes are accessed from the Stock Solutions Preparation (Tecan). Appropriate tubes are barcode labeled and tared. Reagents are dosed (via Chemspeed station or manually) and solubilized by the liquid handler to the desired concentration. Solid reagents can be weighed directly into the reaction tubes.
   b. In the Reactions Screening Platform (Tecan), reaction tubes are provided with magnetic stir bars and placed in an H+P Variomag96 reactor (default stirring is 500 rpm). The reaction block is sealed with a unique lid with individual sealing positions, allowing for liquid dispensing inside each tube even during the incubation process. Each column of the lid is individually connected to argon and a vacuum for an inert atmosphere purge. Stock solutions are placed in their corresponding H+P Variomag48 reactor block, provided with a unique lid that allows an inert atmosphere, and magnetically stirred once the experiment is started. Additional solvents can be loaded onto the Tecan deck.
   c. The experimental protocol is uploaded to the EPS software and initiated. EPS commands the instrument and automatically generates the corresponding Tecan scripts for dispensing, purging, and aliquoting, while also controlling the reaction blocks and cryostat.

6. Reaction analysis: Reaction monitoring can be done during or after incubation. An aliquot is aspirated to individual filters and directly loaded into a 96-well microplate that is transferred to an HPLC-MS.

7. Reporting: HPLC-MS results are automatically transferred to Autolab for quick visualization, exported to JMP for DoE analysis, and exported to the eLN for a final report.

Our HTE plan toward 25 was encouraged by literature reports on the enantioselective fluorination of aldehydes in the presence of chiral amines. Nonetheless, minimal precedent existed for the diastereoselective introduction of fluorine α to an aldehyde to obtain a quaternary center in such a congested steric environment. Fluoroalcohols 26a and 26b were originally prepared via an oxidation/fluorination/reduction sequence (Scheme 7). One-pot formation of aldehyde pyrrolidine imine, followed by treatment with Select-Fluor, afforded fluoraldehydes 29a and 29b. Reduction with NaBH₄ provided a 7:1 mixture of amino alcohol diastereomers (26a/26b), delivering 5% isolated yield of
the desired diastereomer 26b. All attempts at direct fluorination of the enamine derived from aldehyde 28 favored formation of the undesired diastereoisomer.42 We hypothesized that a chiral amine in combination with a fluorinating agent might shift the selectivity of the fluorination (Figure 14). In the absence of a chiral amine, formation of diastereomer 26b was detected with Select-Fluor (30a), while other fluorinating agents delivered exclusively undesired 26a or led to decomposition. Focusing on Select-Fluor, we then studied chiral amines as organocatalysts to further improve diastereoselectivity. Under most conditions tested, slow conversion and significant amounts of side products were observed; fortunately, promising results were seen with 0.3 equiv of amines 31a, 31c, 31d, and 31e. Further screening showed that stoichiometric quantities of the chiral agent afforded the best results, with 31e being optimal.42

A wide selection of solvents was also screened for the diastereoselective fluorination (Table 5). Alcoholic solvents (entries 2, 8, and 12) performed best, delivering up to 90% conversion and 3:1 selectivity for the desired diastereomer. Other solvents failed to provide a stable imine intermediate. Alcoholic solvents were rerun on a 5 g scale, confirming trifluoroethanol as the optimal choice. Scaled-up reactions using MeOH or EtOH gave mixtures of desired product with a significant amount of byproduct. Temperature was found to have little impact on diastereoselectivity or reaction rate. Above 35 °C, larger amounts of side products were obtained regardless of the solvent used. On the other hand, trace water slowed down the reaction rate:

Figure 14. Selected fluorination agents and chiral amines.

Table 5. Solvent Selection Screening

<table>
<thead>
<tr>
<th>entry</th>
<th>solvent</th>
<th>imine conversion</th>
<th>26a (area %)</th>
<th>26b (area %)</th>
<th>ratio 26b/26a</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>dioxane</td>
<td>65%</td>
<td>33</td>
<td>60</td>
<td>1.8</td>
</tr>
<tr>
<td>2</td>
<td>EtOH</td>
<td>95%</td>
<td>22</td>
<td>43</td>
<td>2.0</td>
</tr>
<tr>
<td>3</td>
<td>ACN</td>
<td>75%</td>
<td>42</td>
<td>48</td>
<td>1.1</td>
</tr>
<tr>
<td>4</td>
<td>toluene</td>
<td>25%</td>
<td>60</td>
<td>13</td>
<td>0.2</td>
</tr>
<tr>
<td>5</td>
<td>NMP</td>
<td>64%</td>
<td>59</td>
<td>22</td>
<td>0.4</td>
</tr>
<tr>
<td>6</td>
<td>DMA</td>
<td>69%</td>
<td>54</td>
<td>27</td>
<td>0.5</td>
</tr>
<tr>
<td>7</td>
<td>MTBE</td>
<td>40%</td>
<td>38</td>
<td>40</td>
<td>1.1</td>
</tr>
<tr>
<td>8</td>
<td>MeOH</td>
<td>94%</td>
<td>30</td>
<td>57</td>
<td>1.9</td>
</tr>
<tr>
<td>9</td>
<td>IPA</td>
<td>73%</td>
<td>27</td>
<td>44</td>
<td>1.6</td>
</tr>
<tr>
<td>10</td>
<td>DCM</td>
<td>67%</td>
<td>32</td>
<td>57</td>
<td>1.8</td>
</tr>
<tr>
<td>11</td>
<td>MeTHF</td>
<td>57%</td>
<td>40</td>
<td>53</td>
<td>1.3</td>
</tr>
<tr>
<td>12</td>
<td>trifluoroethanol</td>
<td>96%</td>
<td>20</td>
<td>61</td>
<td>3.1</td>
</tr>
<tr>
<td>13</td>
<td>THF</td>
<td>75%</td>
<td>38</td>
<td>54</td>
<td>1.4</td>
</tr>
<tr>
<td>14</td>
<td>EtOH–Tol (1:5)</td>
<td>64%</td>
<td>30</td>
<td>43</td>
<td>1.4</td>
</tr>
<tr>
<td>15</td>
<td>MeOH–Tol (1:5)</td>
<td>94%</td>
<td>38</td>
<td>42</td>
<td>1.1</td>
</tr>
<tr>
<td>16</td>
<td>EtOH–DCM (1:5)</td>
<td>93%</td>
<td>23</td>
<td>51</td>
<td>2.2</td>
</tr>
<tr>
<td>17</td>
<td>MeOH–DCM (1:5)</td>
<td>94%</td>
<td>30</td>
<td>57</td>
<td>1.9</td>
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<tr>
<td>18</td>
<td>MeOH–MTBE (1:5)</td>
<td>59%</td>
<td>38</td>
<td>53</td>
<td>1.4</td>
</tr>
<tr>
<td>19</td>
<td>MeOH–MeTHF (1:5)</td>
<td>81%</td>
<td>38</td>
<td>52</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Conversion and area % were calculated by HPLC. aHeterogenous suspension. bSticky oily solid. dHomogeneous solution.

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almost full conversion was observed after 24 h when using freshly distilled trifluoroethanol or technical grade solvent, but reaction time could be further reduced to 4 h when dry solvent was used.43

Following our results, epimeric mixture 28 was fluorinated on a 1.6 mol scale using Select-Fluor (30a), d-proline (31e), and dry trifluoroethanol as solvent to provide 29a/29b in 90% yield and 1:7 selectivity. Amine 31e and Select-Fluor (30a) byproducts were removed by extraction, and salts were separated by filtration. After several unsuccessful attempts to isolate the desired isomer 29b by crystallization or bisulphite adduct formation, the crude fluoroaldehyde mixture 29a/29b was directly transformed into the desired chiral amino alcohol 26b by reduction with NaBH₄ in EtOH. After 2 h, 98%
conversion was achieved, and no isomerization was observed under the reaction conditions. It was confirmed that lowering the reaction temperature to 0 °C during the addition of NaBH₄ afforded better results than running the chemistry at room temperature.⁴²

In summary, the use of HTE allowed the rapid exploration and development of an efficient, practical, and scalable fluorination reaction mediated by D-proline in trifluoroethanol, generating a congested quaternary stereocenter in high yield and with good diastereoselectivity. The process was able to be scaled to kilogram quantities wherein the trifluoroethanol could be recovered and recycled. The optimization of the α-fluorination procedure was critical to shift the reaction diastereoselectivity from 1:7 to 7:1.

4.5. Case Study #5: HTE for Optimization of Metallophotoredox Catalysis Reactions. The resurgence of visible light photocatalysis over the past decade has enabled a multitude of unique and traditionally challenging bonds to be formed readily.⁴⁴ As a result, photocatalytic methods have been incorporated in the synthesis of pharmaceutically relevant compounds with increasing frequency.⁴⁵ Of recent interest are methods that merge photoredox catalysis with transition metal oxidation of NiII to NiIII, driving facile reductive elimination of resultant carbon-centered radicals in turn participate in Ni-catalyzed couplings with aryl halides (outlined in Figure 16c).

During our initial foray into metallophotoredox catalysis, we found that optimization was required to effectively adapt conditions reported in the literature to pharmacetically relevant substrates. Given the inherent complexity of these multicatalytic systems, we decided to leverage the power of rational HTE to accelerate optimization work and began development of a standardized platform.

In order to develop an effective platform, we elected to study a relatively complex HAT-metallophotoredox-catalyzed α-hydroxyl arylation method under development by MacMillan and co-workers (Figure 17).⁵⁰ The mechanistic hypothesis for this method was similar to that proposed for the α-amino/oxo arylation of amines and ethers (see Figure 16c). We envisioned that introduction of a Lewis acid to the system would promote hydrogen atom abstraction at the α-C–H position of alcohols, generating carbon-centered radicals that would participate in Ni-catalyzed couplings with aryl halides. We hypothesized that judicious choice of a Lewis acid additive could suppress any undesired C–O coupling. In order to determine the optimal Lewis acid, we turned to HTE.

HTE was carried out in 96-well Para-dox photocatalyst optimization blocks with 1 mL glass vial inserts, under a positive pressure nitrogen atmosphere (M Braun Glovebox).⁵¹ The reaction scale was fixed at 10 μmol of aryl halide in about 50 μL of reaction solvent to minimize the path length for optimal photon flux (<2 mm).⁵² Dispense steps were carried out via hand-held pipets, following the general procedure outlined below, but minor changes were made in each screen to minimize the number of unit operations:

1. A mixture of Ni precursor and ligand in acetonitrile was aged for at least 15 min to generate the precatalyst complex and then dispensed to the block. The acetonitrile was evaporated using a Genevac vacuum evaporator.
Figure 18. HTE evaluation of Lewis acid additives. [Performed on a 10 μmol scale with photocatalyst 32 (0.5 mol %), NiBr$_2$·Me$_4$Phen 33 (0.5 mol %), quinuclidine 34 (100 mol %), aryl halide 35 (1.0 equiv), alcohol 36 (5.0 equiv), and additive (1.5 equiv), in DMSO (0.25 M).] Visualization prepared in Tableau.

Figure 19. HTE evaluation of inorganic bases. [Performed on a 10 μmol scale with photocatalyst 32 (0.5 mol %), NiBr$_2$·Me$_4$Phen 33 (0.5 mol %), quinuclidine 34 (25 mol %), aryl halide 35 (1.0 equiv), alcohol 36 (5.0 equiv), ZnX$_2$ (1.5 equiv), and base (1.0 equiv) in DMSO (0.25 M) with and without water (10.0 equiv).] Visualization prepared in Excel.
2. Parylene coated magnetic tumble stir bars (V&P Scientific, VP 711D-1) were dispensed to the block using a stir bar dispensing tool (V&P Scientific, VP 711A-96-AS-1).

3. A mixture of aryl halide, coupling partner, photocatalyst, quinuclidine, base, and additive was prepared in each reaction solvent and dispensed to the block.

4. Two rubber mats and one PFA film were screwed down with a metal lid to prevent solvent evaporation.

5. Illumination was carried out with Lumidox 470 nm 96 LED arrays set to 30 mA output, with tumble stirring (V&P Scientific, VP 710E5), under a positive pressure nitrogen atmosphere, for 24 h. Under these conditions, it was typical for the block temperature to reach 35 °C upon equilibration.

6. A mixture of internal standard in acetonitrile and DMSO was added to the block, and the diluted mixtures were sampled into an analysis block containing acetonitrile and analyzed by UPLC-MS.

In the first array, we evaluated 23 Lewis acid additives in four solvents (dimethyl sulfoxide, acetonitrile, acetone, and ethyl acetate), alongside four control experiments containing no additive. Of the four solvents screened, appreciable reaction rates were observed only in DMSO. The distributions of the α-hydroxyl arylation product and C−O coupling product in DMSO are presented in Figure 18. In the absence of additive, C−O coupling proved to be the predominant pathway. However, the nature of the Lewis acid made a significant impact on product distribution, with zinc salts serving to suppress the competitive C−O coupling pathway and yielding roughly 45%.

"Performed on a 300 μmol scale with photocatalyst 41 (0.2 mol %), NiBr₂-Me₆Phen 33 (1.5 mol %), quinuclidine 34 (30 mol %), aryl halide 35 (1.0 equiv), alcohol 36 (5.0 equiv), ZnCl₂ (1.5 equiv), and K₃PO₄ (1.0 equiv) in DMSO (0.25 M)."
of the desired product. The mass balance laid in the formation of dehalogenation and homocoupling byproducts, 39 and 40, respectively.

We next shifted our focus to reducing the dehalogenation and homocoupling byproducts through prudent selection of base, as earlier catalyst loading experiments indicated a positive correlation between the stoichiometry of the HAT catalyst, quinuclidine, and byproduct formation. We therefore reduced the quinuclidine loading to 25 mol % and evaluated 23 inorganic bases in the presence of two zinc salts, in two solvent systems. Control experiments with quinuclidine were also included in the study. We were pleased to uncover two inorganic bases, $\text{K}_3\text{PO}_4$ and $\text{NaOH}$, that reduced byproduct formation and yielded roughly 60% of the desired product (Figure 19).

At this point, we attempted scale-up experiments using cylindrical vials illuminated by a 40 W blue Kessil lamp and found that the photocatalyst loading needed to be reduced from 0.5 to 0.2 mol % and Ni catalyst loading increased from 0.5 to 1.5 mol % in order to effectively reproduce our findings with $\text{K}_3\text{PO}_4$. Then, through optimization of the photocatalyst 32 structure, we improved yields further, resulting in the isolation of 75% of the $\alpha$-hydroxyl arylation product 37 (Scheme 8).

With an effective and reproducible workflow for custom HTE in place, we next turned to developing standardized, predispensed metallophotoredox catalysis kits for distribution across the Merck chemistry network. On the basis of our survey of the literature, we established four kits categories: metallophotoredox C$\equiv$O, C$\equiv$N, C$\equiv$C, and HAT C$\equiv$C (Figure 20).

For each category, we selected the optimal photocatalysts for robotic dispensing of stock solutions into 24-well kits and Ni catalysts for robotic dispensing into glass vials (Chemspeed Swing then Genevac). The selections presented here represent our second round of kit designs, as it was necessary to update the first round within a short time span to reflect rapid advances in the field. In particular, we incorporated dicyanobenzene-based donor–acceptor fluorophores such as 4CzIPN and 4DPAIPN into the kit designs, as they had been demonstrated to be competent organophotocatalysts in Ni photoredox catalysis reactions. Finally, optimal photocatalyst loadings were determined to be between 2 and 5 mol % when illuminating 1 mL glass vials with 50 μL fill volumes using Lumidox 96 LED arrays.

Once the kit designs were complete, the photocatalysts were dissolved in acetonitrile and plated. The Ni complexes were generated in acetonitrile from NiBr$_2$·glyme and the appropriate ligands and then dispensed to vials. Upon evaporation of the acetonitrile and stir bar addition, kits and vials were packaged and stored in purge boxes under positive pressure nitrogen atmosphere.

To execute experiments, end-users prepared mixtures of starting material, base, and additive (if applicable) in each solvent and dispensed to the photocatalyst plates. They then prepared mixtures of each Ni complex in each solvent through redissolution of the predispensed vials; these mixtures were then added to the plate. Users sealed, illuminated, and agitated the blocks under an inert atmosphere to ensure optimal reaction performance. Finally, dilution and sampling steps were carried out.

We have performed multiple successful optimizations using these kits which are beyond the scope of this publication. In general, successful results were initially scaled up in PennOC photoreactors$^{56}$ and then gradually moved to photoflow reactors for larger scale syntheses.$^{57}$ Some photocatalyst and Ni loading optimization was needed when moving between photoreactors due to differences in light wavelength, bandwidth, and intensity.

We have presented an effective workflow for optimization of metallophotoredox catalysis reactions through HTE and successfully applied this to the optimization of a novel $\alpha$-hydroxyl arylation method. We then demonstrated the scalability of these results by a factor of 30. Finally, we leveraged these learnings to develop standardized metallophotoredox catalysis kits for wide distribution across the MSD chemistry network. It is important to note that new reports have rendered it necessary to update kit designs frequently, and a continuation of this trend is expected due to the emerging nature of the field. Also inevitable is the arrival of new and improved photoreactors, which may necessitate further updates of kit designs. Nevertheless, the described metallophotoredox HTE workflow can be employed as a general framework for future optimization, even as new methods, mechanistic insights, and tools emerge.

4.6. Case Study #6: Application of Miniaturized Design of Experiments Studies to the Optimization of Methyl Ester Hydrolysis. As part of a research project at Pfizer, our group was tasked with the optimization of an ester hydrolysis to afford acid 43 (Scheme 9). The starting material 42, in addition to the methyl ester moiety, contains an acid-labile oxetane ring and a base-sensitive nitrile group that undergoes a competitive partial hydrolysis to afford amide impurity 44. This case study describes our efforts to optimize the hydrolysis of 42 under basic conditions while minimizing the amount of 44 through the use of Design of Experiments (DoE) and our Unchained Laboratories Freeslate Junior automated reaction screening platform.

Miniaturized DoE studies are carried out at Pfizer on an Unchained Laboratories Freeslate Junior platform that is housed in a purge box with oxygen levels <20 ppm. The automation platform is controlled by the Laboratory Execution and Automation (LEA) software. Experiments are performed in 8 mL vials (Wheaton 224804) which are capped with a PTFE/silicone septum-containing cap (Wheaton 240842SP) and placed on a 6 × 8 aluminum block (Unchained Laboratories F157404) on the Freeslate Junior’s main deck. Each vial contains a stick stir bar (V&P Scientific, VP 775-20).$^{35}$ The total reaction volume is fixed at 4 mL per vial to eliminate the fill volume as an uncontrolled variable in the DoE study. This total reaction volume represents a compromise of both maintaining
efficient mixing and having sufficient volume to enable 12−15 samples per reaction. Sampling is carried out in an automated fashion by the Freeslate Junior, and samples are quenched into 2 mL UPLC vials containing a flea stir bar and quench solution. The quench plates are maintained at 20 °C on the deck and stirred at 200 rpm to ensure complete reaction quenching. Our miniaturized DoE workflow is shown in Figure 21.

Prior to executing the DoE experiments, we conduct familiarization runs to reproduce previous results and determine potential variables and ranges. Once these variables and ranges for the DoE study have been explored and agreed upon with the process chemist, a set of experiments is generated using DoE software, such as Design Expert. The choice of DoE largely depends upon the study scope and material availability. Calculations for the amount of reactants, reagents, and solvents are performed in Excel or by means of the DoE import tool in Library Studio software. Solid dispensing into 8 mL vials can be performed manually or, preferably, using Mettler-Toledo’s Quantos QB5 platform. Liquid dispensing can be carried out using electronic repeater pipettes or, also preferably, in an automated fashion with the Freeslate Junior. The entire DoE recipe can be assembled in Unchained Laboratories’ Library Studio, including liquid reagent dispensing, stirring rates, heating/cooling, and sampling at desired intervals. Once the experiments have been carried out, the samples are analyzed by UPLC (if a chromophore is present), and the UPLC data is processed using Waters’ Empower software. The UPLC data are fed back into Design Expert and TIBCO Spotfire to generate plots and help visualize the results. On the basis of the DoE results, additional experiments, such as a second DoE or one-off trials, may be planned in 8 mL vials with fewer variables and/or narrower ranges to pinpoint the optimal conditions for the desired transformation. After work in 8 mL vials has been completed and all of the information is in hand, the team decides on a select group of conditions to scale up as confirmation runs in either an Easymax or Systag reactor (100 mL scale). These scale-up experiments may be replicates of previous small-scale runs or may be conditions that, according to the statistical model predictions, should give an adequate outcome. If the output from those scale-up runs is satisfactory, the information is passed on to the process chemist for further evaluation and optimization. Any discrepancies in the results between 8 mL vials and larger scale vessels must be addressed at this stage and may point to factors such as mixing sensitivity, especially for heterogeneous or biphasic reactions.

Our process chemistry group had previously developed a protocol for the methyl ester hydrolysis of 42 using 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) as base.59,60 The level of amide impurity 44 produced from this protocol (5−10%) could not be reduced to acceptable levels via recrystallization, and chromatographic purification was required. As a result, alternative conditions were evaluated to minimize the amount of 44 and avoid the need for chromatography to support larger scale campaigns.

Since acidic conditions for ester hydrolysis were precluded by the oxetane ring, our first step was screening for alternative basic conditions that completely suppressed or minimized the amount of amide impurity 44. Thus, high-throughput screening of 12 inorganic bases (carbonates, bicarbonates, phosphates, and hydroxides) identified a THF/DMSO/water combination with NaOH as a promising lead. Further screening on a larger scale with variable amounts of NaOH (2−5 equiv) and water (5−20 equiv) in a THF/DMSO mixture (16 volumes and 6% by volume, respectively) afforded incomplete reactions and, in some cases, very thick slurries that prevented efficient mixing. Side-by-side comparison with the original conditions in aqueous MEK and TBD as base showed no clear advantage to the new conditions. In view of these results and process chemistry’s familiarity with the TBD/aqueous MEK process, a decision was made to optimize this protocol using DoE.

Two DoE studies were executed in a sequential approach to examine four variables that, on the basis of preliminary work by our process chemistry group, could provide guidance of parameters to reduce production of 44. The four variables...
plate-to-plate variability, as well as to test the lack of experimental repeatability, temperature blocking effects, and interactions. The goal of the initial design was to check the validity of initial parameter ranges and to examine parameter main effects and interactions. The outcomes from this first DoE were the following:

1. All variables were significant in that they influenced the amount of amide impurity.
2. Replicates data revealed excellent reaction reproducibility.
3. Desired product formation was favored at high temperature, high base, high water equiv, high concentration, and longer reaction time. See Figure 22 for contour plots.
4. Unfortunately, the same conditions that favored acid 43 formation also favored amide impurity 44 formation at the expense of 43.
5. In general, for conversions in the 50–60% range, the amide impurity level remained at ≤1%, especially at lower temperature (20–40 °C).
6. Considerably faster reactions to acid 43 were in general observed at 60 °C than at lower temperatures but also provided much higher amide 44 levels (up to 61% UPLC AP after 25 h).

From the data in this study, it was concluded that complete suppression of amide 44 formation would be unlikely and that a compromise would have to be reached between conversion to acid 43 and the level of 44. As a result, a second DoE using a face-centered central composite design was executed to optimize the reaction with the following guidelines:

1. Targets were no more than 5% for both methyl ester 42 and amide 44, as both impurities could be purged up to those levels during workup to meet specs.
2. Due to the longer timelines to conduct IPC sampling and analysis in the plant, variable ranges were chosen to have a broad window of time (several hours) in which high conversion to product could be obtained while, at the same time, the amount of amide 44 would remain below the 5% threshold.

For this second DoE, narrower parameter ranges were selected as follows: TBD, 1–2 equiv; water, 10–50 equiv; MEK, 5–15 volumes; temperature, 20–60 °C. Each reaction was sampled eight times at 0.8, 1.4, 2.5, 4.6, 6.7, 10.8, 16.9, and 25.1 h and quenched into pH 5.4 buffer. The replicates were chosen within and across plates to allow evaluation of experimental repeatability, temperature blocking effect, and plate-to-plate variability, as well as to test the lack of fit of the model. The goal of the initial design was to check the validity of initial parameter ranges and to examine parameter main effects and interactions.

Table 6. Summary of Reaction Conditions for the Four Scale-up Confirmation Experiments

<table>
<thead>
<tr>
<th>experiment</th>
<th>T (°C)</th>
<th>TBD (equiv)</th>
<th>water (equiv)</th>
<th>MEK (vol)</th>
<th>predicted end point window (h)</th>
<th>sampling time (h)</th>
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</thead>
<tbody>
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<td>2</td>
<td>30</td>
<td>5</td>
<td>5.5–7</td>
<td>1, 3, 4, 5, 5.5, 6, 6.5, 7, 8, 10, 14, 18, 20, 24, 28</td>
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<tr>
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<td>25</td>
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<td>35</td>
<td>7.5</td>
<td>13–17</td>
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<td>2</td>
<td>20</td>
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<td>14–20</td>
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<tr>
<td>model prediction</td>
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<td>20</td>
<td>7.5</td>
<td>13.7–16.5</td>
<td>2, 4, 8, 10, 12, 13, 14, 15, 16, 17, 18, 20, 24, 28</td>
</tr>
</tbody>
</table>

were initially incorporated into a first two-level full factorial DoE with replicates at center and two other design conditions for a total of 24 reactions split into four 6 × 8 aluminum plates to accommodate 8 mL vials. The variables and ranges in this first DoE were as follows: TBD, 1–3 equiv; water, 10–50 equiv; MEK, 5–15 volumes; temperature, 20–60 °C. Each reaction was sampled eight times at 0.8, 1.4, 2.5, 4.6, 6.7, 10.8, 16.9, and 25.1 h and quenched into pH 5.4 buffer. The replicates were chosen within and across plates to allow evaluation of experimental repeatability, temperature blocking effect, and plate-to-plate variability, as well as to test the lack of fit of the model. The goal of the initial design was to check the validity of initial parameter ranges and to examine parameter main effects and interactions.

The outcomes from this first DoE were the following:

1. All variables were significant in that they influenced the amount of amide impurity.
2. Replicates data revealed excellent reaction reproducibility.
3. Desired product formation was favored at high temperature, high base, high water equiv, high concentration, and longer reaction time. See Figure 22 for contour plots.
4. Unfortunately, the same conditions that favored acid 43 formation also favored amide impurity 44 formation at the expense of 43.
5. In general, for conversions in the 50–60% range, the amide impurity level remained at ≤1%, especially at lower temperature (20–40 °C).
6. Considerably faster reactions to acid 43 were in general observed at 60 °C than at lower temperatures but also provided much higher amide 44 levels (up to 61% UPLC AP after 25 h).

From the data in this study, it was concluded that complete suppression of amide 44 formation would be unlikely and that a compromise would have to be reached between conversion to acid 43 and the level of 44. As a result, a second DoE using a face-centered central composite design was executed to optimize the reaction with the following guidelines:

1. Targets were no more than 5% for both methyl ester 42 and amide 44, as both impurities could be purged up to those levels during workup to meet specs.
2. Due to the longer timelines to conduct IPC sampling and analysis in the plant, variable ranges were chosen to have a broad window of time (several hours) in which high conversion to product could be obtained while, at the same time, the amount of amide 44 would remain below the 5% threshold.

For this second DoE, narrower parameter ranges were selected as follows: TBD, 1–2 equiv; water, 20–40 equiv; MEK, 5–10 volumes; temperature, 20–40 °C. The DoE design was split into five 6 × 8 aluminum plates with replicates at the center and several selected conditions within and across plates for a total of 42 reactions. Automated sampling was carried out as described above with the Freeslate Junior at 1, 2, 4, 6, 8, 10, 16, and 24 h. The experimental results from the second set of reactions are as follows:

1. As in the first DoE, all reactions showed that, at some point, the amount of desired product peaked and then, at extended reaction times, amide 44 started forming in increasing amounts at the expense of desired product 43.
2. Higher temperature and more equiv of both water and base increased the amounts of amide 44.
3. The second round of experiments clearly confirmed that a compromise would have to be reached between conversion and amount of amide impurity.

After analysis of kinetic profiles, three reactions stood out as the most favorable in terms of conversion to product and minimization of amide 44 over an extended time window: these are labeled as experiments 1–3 in Table 6. The reaction end point was defined as ≥90% product and ≤5% ester starting material. The end point windows for experiments 1–3 as predicted by the statistical model were about 1.5, 4, and 6 h, respectively, in which the percentage of acid 43 is ≥90%, whereas amide 44 remains ≤5% (UPLC area %). On the basis of these data, the team decided to proceed with the scale-up of these three conditions. In addition, the statistical model...
predicted a fourth set of conditions that should afford the desired outcome ($\leq 5\%$ and $\leq 44\%$) over an approximately 3 h end point window ($13.7 - 16.5$ h): TBD, 2 equiv; water, 20 equiv; MEK, 7.5 volumes at $25^\circ$C. This set of conditions was also scaled up to confirm the validity of the statistical model.

A summary of the four scale-up confirmation experiments with sampling times is shown in Table 6. The scale-up experiments were carried out in a 100 mL Systag reactor equipped with a mechanical stirrer, a temperature probe, and a Mettler-Toledo Easysampler probe to sample the reactions. Sample frequency was increased during the predicted optimal time windows to keep close track of methyl ester $42$ and amide $44$ levels in those intervals.

The outcomes from these experiments are plotted in Figure 23, in which the kinetic profiles for the 8 mL experiments (circles) have been overlaid with the results from the scale-ups (crosses) to demonstrate reproducibility (experiments 1–3). The fourth plot in Figure 23 displays the results for the statistical model prediction experiment.

Overall, the results for experiments 1–3 show very good reproducibility in going from 8 mL vials to the 100 mL Systag reactor, as demonstrated by the close overlapping of the kinetic profiles for $42$, $43$, and $44$. Other conclusions from the scale-up experiments are as follows:

1. Experiment 1: Slightly slower ester hydrolysis was seen on scale. The observed optimal time window to meet the desired $\leq 5\%$ for both methyl ester $42$ and amide $44$ was very narrow (less than 1 h) and pushed out toward the end of the predicted 5.5–7 h interval. Also, of the four scale-ups, these conditions provided the largest amount of amide $44$ over extended reaction times.

2. Experiment 2: The reaction rates in 8 mL vials and on scale were virtually identical, and the observed end point window closely matched the predicted one ($13–17$ h).

3. Experiment 3: As for experiment 1, a slower reaction was observed on scale, which pushed the end point window out to approximately 19–28 h. At a given time point, these reaction conditions provided the smallest amount of amide $44$ out of experiments 1–3. A possible explanation for the slower reaction rate may be mixing sensitivity in going from magnetic to mechanical stirring, as this was the most concentrated reaction and large amounts of solids were present.

4. Statistical model prediction experiment: A slower-than-expected reaction was also observed in this case, which pushed the end point window for sampling out from the expected 13.7–16.5 h to approximately 22–28 h.

In view of the data collected during scale-up work, a decision was made to select the reaction conditions in experiment 3 as the best compromise between reaction time to meet desired targets for $42$ and $44$ and have an extended time window to allow for sampling and reaction quenching in a pilot plant facility. Optimized reaction conditions to minimize an amide byproduct during a methyl ester hydrolysis have been identified through the use of DoE studies. Even though DoE could not identify reaction conditions that totally suppressed the
generation of amide 44, it directed us toward an experimental domain in which a compromise could be reached between high conversion to the desired acid and slow generation of the amide impurity. Through the combination of preliminary one-off experiments and a first DoE with broad variable range, a second DoE with narrower ranges was designed and executed that led to the identification of three sets of conditions that were scaled up in confirmation runs. In addition, the mathematical model predicted a fourth set of conditions which was also tested on scale. From this series of experiments, a particular set of conditions was identified as a potential replacement to the current process in future campaigns, providing an optimal compromise between reaction rate and amide 44 formation.

5. CONCLUSION

Herein we have presented an overview of how HTE groups in both industry and academia have embraced recent technological and engineering advances to deliver improved insight to reaction discovery and chemical process development. HTE has provided a complementary paradigm toward process development to classical one-factor-at-a-time approaches, enabling an enhanced exploration of chemical space and process variables. When used in the chemical industry, HTE can support a variety of distinct workflows ranging from preclinical development to manufacturing support. To illuminate the impact of HTE across the pharmaceutical industry, we distributed a survey to the chemical technology groups at a number of leading companies. We learned that HTE approaches have been employed to assess over two dozen different bio- and chemocatalytic reaction types, looking at both discrete and continuous reaction variables. In addition to traditional reaction development, HTE has also been deployed to study downstream unit operations such as salt formation and metal scavenging.

We expect the chemical enterprise will place a greater emphasis on HTE techniques in the coming years. Our survey results demonstrate a continued interest in HTE workflows to optimize both the most commonly used transformations in organic synthesis as well as novel reaction paradigms (i.e., photoredox catalysis and C–H activation). They also support greater integration within process chemistry departments, as specialized equipment becomes more user-friendly and dedicated HTE teams begin to promote an end-user approach to basic reaction screening. As the value of HTE becomes more widely acknowledged, we believe its principles will also begin to be applied to other aspects of process development such as reaction workup and product isolation.

An increased focus on HTE and the use of micro- and nanoscale screening will require new tools to maximize insights and accelerate development. Several of our case studies revealed examples of screening hits that could not be effectively reproduced on a large scale. A better understanding of how screening platforms can mimic scaling effects will allow HTE to provide more robust leads. While reaction screening is routinely performed in parallel, reaction analysis remains a more linear endeavor. Adoption of faster or parallel analytics in chemistry laboratories will reduce the cycle time between a hypothesis and results generation. Additionally, the possibility of expanded analytical techniques that would allow for continuous monitoring of multiple reactions in parallel would enable a paradigm shift for experimental design and impact of chemical transformations. As the amount of data increases, the ability to interpret results efficiently and draw appropriate conclusions will require new data management and modeling tools and should also leverage recent advances in machine learning and first-principles modeling. With these holistic improvements to data generation and interpretation, the value of HTE will continue to broaden.

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Notes
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(58) The choice of stir bar was determined after mixing studies carried out in our laboratory that focused on a Bourne reaction as an example of mixing-sensitive reaction. On the basis of the outcome of those studies, we decided to employ this stir bar in all future project work carried out on our Freeslate Junior platform.


(61) The exact sampling times were determined from Polyview data collected by the LEA software.

(62) pH 5.4 buffer (made by mixing 0.1 M aqueous citric acid (44 mL) and 0.2 M aqueous dibasic sodium phosphate (56 mL) and diluting with 100 mL of MeCN) was required to neutralize the base and stop the reaction. Also, this pH was not acidic enough to cause oxetane ring opening.

(63) DoE volumes (L/kg) were selected on the basis of tank capacity in the plant and to avoid multiple runs.