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High-Throughput Experimentation at Domainex

White Paper

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Introduction

High-Throughput Experimentation (HTE) is a powerful technique for reaction optimisation; it enables multiple reaction variables to be assessed in parallel in one single experiment. HTE is beneficial because it minimises the amount of reagents used, which in addition to being time and cost effective, is a greener way of conducting chemistry.[1,2] At Domainex, we have adopted HTE as part of our standard way of working and regularly use it to troubleshoot problematic reactions.

Methods

Preparation of "end-user plates"

The development of "end-user plates" has been fundamental to our HTE platform. Our end-user plates consist of 24 vials (six columns by four rows), where each well contains a unique set of reaction conditions. The vials are available in two different sizes, mL and µL, for which 10 µmol and 2.5 µmol of limiting reagent is used per well respectively (Figure 1). The choice of plate size is decided on a case-by-case basis to align with project needs. Each glass vial in the plate contains the requisite reagent(s) and the end-user plate is stored until it is needed. When a chemist has a reaction they would like to optimise, they can take one of the pre-prepared plates from storage, and add their reagents, solvent, and a base if needed. The plate can be prepared in the glovebox (Figure 2) and then sealed before removal, so even air-sensitive reactions can be screened in a plate-based format. Additionally, decarboxylative photoredox coupling reactions can be performed in a high-throughput fashion using Lumidox II photochemistry apparatus (Figure 3).

Figure 1: A 24 x 1 mL well-plate (left) and a 24 x 200 µL well-plate (right)

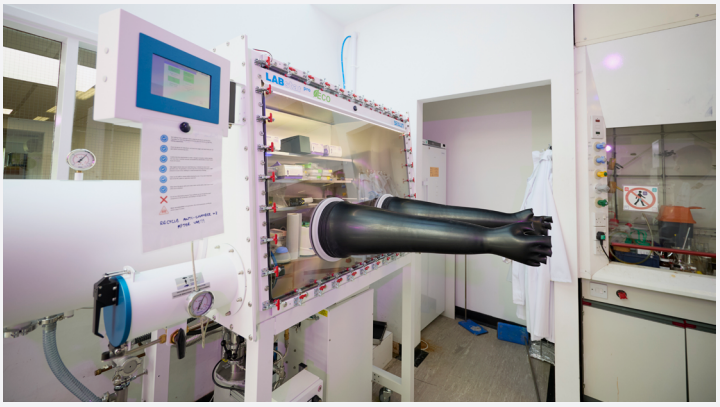


Figure 2: MBRAUN LABstar pro glovebox, used for setting up plate-based reactions

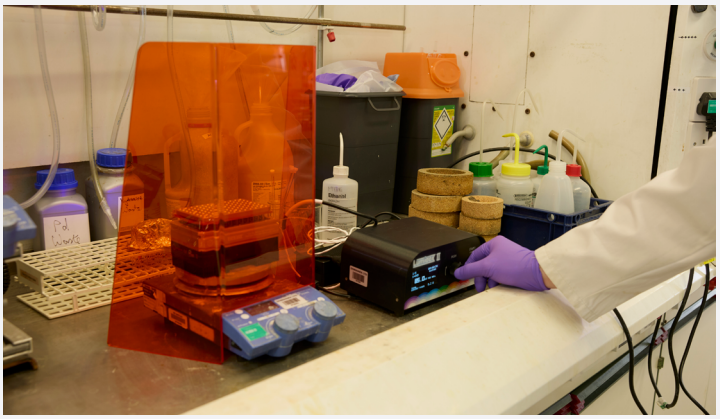


Figure 3: Lumidox II photochemistry apparatus, used here for a decarboxylative photoredox coupling reaction

High throughput data analysis using PyParse

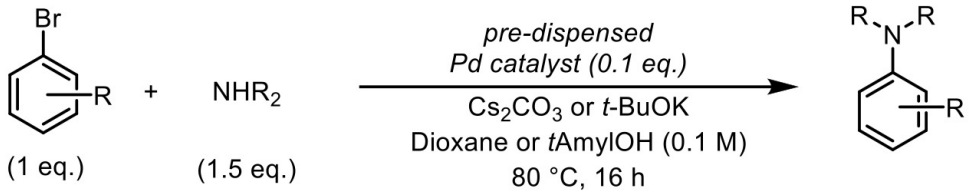
Once the plate-based reactions have been left for the requisite time, UPLC-MS data is obtained for each reaction sample. To streamline the analysis of all UPLC-MS data that has been collected for our plate-based reaction optimisation, we use a tool known as PyParse. PyParse is a Python script that compares the UPLC-MS data with a list of expected compounds, provided by the user in a .csv file.[3] By calculating the expected mass ions for each compound, and incorporating its own specially written algorithm to remove false positives, PyParse then matches up the right peak with the right compound. Achieving global usage and recognition, this free-to-use tool differentiates itself through its user-friendly interface, variety of graphical outputs and speed. For instance, a full 384-well plate can be analysed in just 4 minutes, compared to manual analysis which could take around 10 hours!

Validation Plates

Before new end-user plates are put into circulation for use on client projects, they are validated using a model substrate. This is to confirm that the chosen variety of reaction conditions, already well precedented in the literature, work in a plate-based format. This work also enables optimisation of the design and setup of the end-user plate itself.

Case Studies illustrating the benefits and efficiency of HTE approach

Case Study 1: C-N Buchwald-Hartwig Cross-Coupling



Scheme 1. The C-N Buchwald-Hartwig cross coupling reaction to be optimised.

A key C-N bond formation using a Buchwald-Hartwig cross coupling showed only trace amounts of desired product. The C-N bond could instead be introduced using an S_NAr approach, but this would have added an extra three steps to the synthetic route. A 24 x 1 mL vial-plate screening was conducted using six different palladium catalysts, two different bases, and two different solvents (Scheme 1). Interestingly, only one of the 24 different sets of conditions gave any product, which were BippyPhos, DAB-Pd-MAH, and Cs_2CO_3 in dioxane (Figure 4). The heatmap is shaded by the ratio of the UPLC-MS peak area of the desired product to the peak area of the internal standard, then normalised. This means that the best well is denoted by a "1.0", and wells with a zero would indicate no product formed. In this example, many experiments would have needed to have been conducted – one after another - to identify a working set of

reaction conditions if a single-experiment screening approach had been used. The reaction conditions were scaled up to 100 mg (of aryl bromide), yielding the desired product in 22% yield and reducing the synthetic route by three steps.

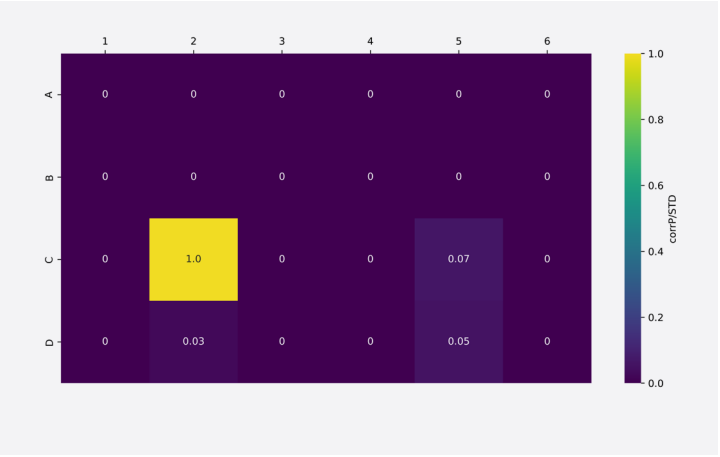
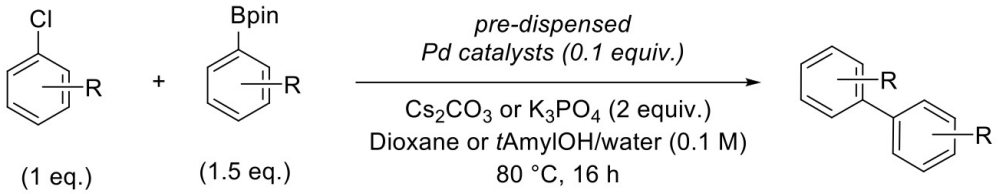


Figure 4: Heatmap for the C-N Buchwald-Hartwig cross-coupling reaction, as described in Scheme 1.

Case Study 2: Suzuki-Miyaura Cross-Coupling



Scheme 2. The Suzuki-Miyaura cross coupling reaction to be optimised.

A Suzuki-Miyaura cross-coupling reaction between an aryl chloride and an aryl boronic ester fragment containing a benzamide had a low (19%) yield with a very messy reaction profile (Figure 5). A Suzuki-Miyaura end-user plate containing six different palladium catalysts, two different solvents, and two different bases was used to optimise the reaction. It was found that the reaction was quantitative with a very clean reaction profile with XPhos Pd G3 and K_3PO_4 in *tert*-amyl alcohol (Figure 6). When these conditions were scaled up to 100 mg of aryl chloride, a 97% yield was achieved with no purification needed (Figure 5 and Figure 6). This was a significant improvement that reduced the time required to synthesise sufficient quantities of this key intermediate.

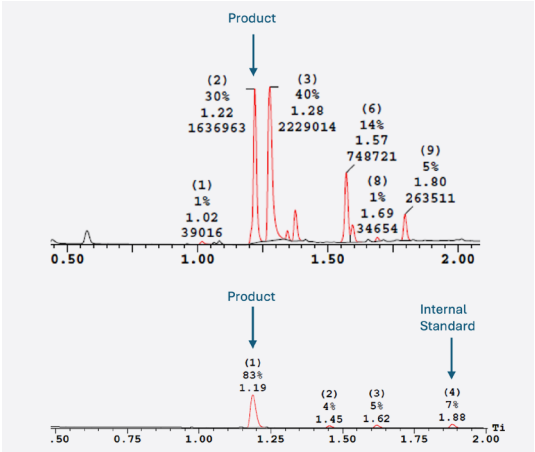
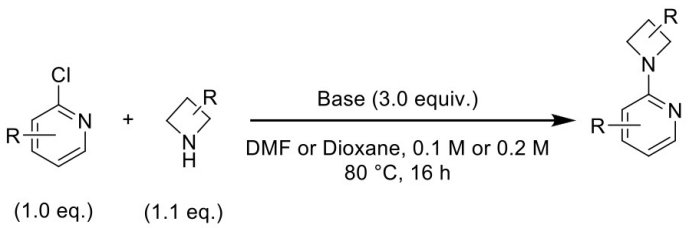


Figure 5: Top: Original UPLC reaction profile for the Suzuki-Miyaura cross coupling reaction described in Scheme 2, Bottom: UPLC reaction profile using the optimised reaction conditions identified by HTE.

Figure 6: Heatmap for the Suzuki-Miyaura coupling reaction, as described in Scheme 2.

Case Study 3: S_NAr reaction



Scheme 3. The S_NAr reaction to be optimised..

A key intermediate to be used for a Direct-to-Biology library synthesis[4] was to be constructed using an S_NAr reaction of an azetidine unit with a heteroaryl chloride (Scheme 3). Literature yields for this type of S_NAr reaction are highly variable, with numerous different conditions used. Given that one of the starting materials was precious, and there was a need to deliver this intermediate quickly, HTE was turned to as a first point of call to rapidly identify the best conditions. A plate was constructed with six different bases and two different solvents, run at two different concentrations. The use of triethylamine in DMF at 0.1 M had a quantitative peak-to-peak reaction profile (Figure 7). This was in stark contrast to reaction conditions using Cs_2CO_3, which showed a complex reaction profile (Figure 7). The triethylamine conditions were scaled up to >3 g of heteroaryl chloride and consistently gave quantitative yields, enabling a straightforward purification by trituration. The use of HTE saved significant time and resources, making the most of a limited quantity of a precious starting material.

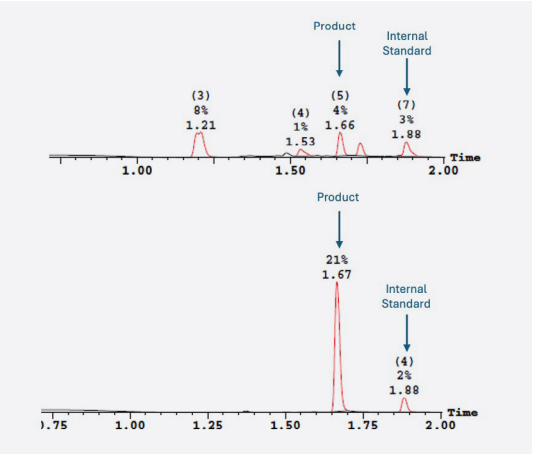


Figure 7. Comparison of UPLC profiles for Cs_2CO_3 conditions (top) with optimised triethylamine conditions (bottom) for the S_NAr reaction described in Scheme 3.

Conclusions

Our HTE platform enables us to screen a range of reaction conditions more rapidly and use fewer starting materials (particularly important when these are expensive or only available in limited quantities) compared to using the sequential, single experiment approach and facilitates the optimisation of reactions in less time. Identifying better reaction conditions not only improves the yields of reactions but also simplifies the purification and isolation of compounds, bringing significant time savings. Even when a reaction cannot be optimised, this can be identified quickly and alternative routes are investigated sooner expediating compound synthesis.

References

1. Shevlin, M. Practical High-Throughput Experimentation for Chemists. *ACS Med Chem Lett* **8**, 601–607 (2017).

2. Caldentey, X. and Romero, E. High-Throughput Experimentation as an Accessible Technology for Academic Organic Chemists in Europe and Beyond. *Chemistry–Methods* **3**, (2023).

3. Mason, J., Wilders, H., Fallon, D. J., Thomas, R. P., Bush, J. T., Tomkinson, N. C. O., and Rianjongdee. F. Automated LC-MS analysis and data extraction for high-throughput chemistry. *Digital Discovery* **2**, 1894-1899 (2023).

4. https://www.domainex.co.uk/services/direct-to-biology-d2b

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