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ORGANIC CHEMISTRY

A platform for automated nanomole-scale reaction screening and micromole-scale synthesis in flow

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The scarcity of complex intermediates in pharmaceutical research motivates the pursuit of reaction optimization protocols on submilligram scales. We report here the development of an automated flow-based synthesis platform, designed from commercially available components, that integrates both rapid nanomole-scale reaction screening and micromole-scale synthesis into a single modular unit. This system was validated by exploring a diverse range of reaction variables in a Suzuki-Miyaura coupling on nanomole scale at elevated temperatures, generating liquid chromatography–mass spectrometry data points for 5760 reactions at a rate of >1500 reactions per 24 hours. Through multiple injections of the same segment, the system directly produced micromole quantities of desired material. The optimal conditions were also replicated in traditional flow and batch mode at 50- to 200-milligram scale to provide good to excellent yields.

In drug discovery programs, it is critical to rapidly synthesize project compounds with the potential to become new therapies, as well as to minimize time spent on ultimately nonoptimal analogs. As such, the application of new synthetic chemistry technologies can play a central role in the accelerated discovery of pharmaceutical agents. High-throughput experimentation (HTE) and flow chemistry (*1*) are enabling technologies (*2*) that often sit at opposite ends of the synthetic scale, with the former encompassing hundreds of micromole-scale batch-type reactions for optimization of conditions and the latter facilitating efficient bulk production of single compounds under wide temperature and pressure ranges (*3*). Although each technique has been advocated as a tool to expedite the drug discovery process, questions remain as to whether this has actually occurred, particularly as the costs involved with this endeavor continue to escalate (*4*). In the earliest phase of a medicinal chemistry program, materials are often a limiting factor, and to increase the coverage of chemical space within a screening campaign, it is necessary to decrease the scale of experimentation. Advanced liquid handling technologies and exclusion of air and moisture in glovebox environments have enabled robust reaction screens on >1-mg quantities of material at 50- to 100-µl volume per reaction. It has therefore become routine to run screens of hundreds of reactions that, in combination with high-throughput analytics such as ultra-performance liquid chromatography–mass spectrometry (UPLC-MS), achieve a rapid turnaround from design all the way through to results in 2 to 3 days, drastically altering the cost/benefit analysis of prospective reaction screening. This approach expands the potential structural diversity of investigated compounds and saves time and resource investment downstream in the development phase if efficient chemistries discovered through HTE are already in place (*5*).

In considering how to further leverage these methods, we were particularly inspired by Dreher, Cernak, and co-workers at Merck, who in a seminal publication provided an elegant solution to enable chemistry at nanomole scale using equipment and technology from biological-assay screening (*6*). Iterative reaction screening in 1000-nl volumes successfully optimized a Pd-mediated Buchwald-Hartwig coupling to yield druglike fragments: 1536 reactions were evaluated in 2.5 hours with as little as 0.02 mg per reaction. However, despite this impressive work, there were still several limitations of this plate-based approach such as the need for nonvolatile solvents (e.g., dimethyl sulfoxide), the absence of heating to avoid solvent evaporation, and application of low-resolution mass spectrometry for analysis. We endeavored to develop a synthetic platform that would overcome these limitations, merging the best attributes of these approaches to screen large numbers of reactions at nanomole scale in a flow system under diverse solvent, temperature, and pressure conditions with the subsequent ability to synthesize hundreds of micromoles for possible biological testing in a highly automated fashion.

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The key objectives were to develop a fully automated system for HTE screening with flow chemistry technology that would (i) integrate inline high-resolution LC-MS analysis for real-time reaction monitoring; (ii) use diverse volatile and nonvolatile solvents; (iii) use ~0.05 mg of substrate per reaction to enable broad parameter space exploration with minimal material consumption; (iv) enable the preparation and analysis of up to 1500 reaction segments in a 24-hour period; (v) establish the capacity of the platform to directly scale up preferred conditions via multiple injections to produce 10- to 100-mg quantities of a specific compound; and (vi) show translation of nano-HTE conditions to both larger-scale batch and flow synthesis.

Solvent variation in flow HTE

Despite the advances in flow chemistry technology, its implementation for high-throughput reaction screening with multiple discrete (e.g., catalyst, ligand, base) and continuous (e.g., temperature, residence time, pressure) variables has been limited to date (*7*, *8*). Furthermore, all flow reaction screening systems have required the preparation of reagent stock solutions when solvent, a key reaction variable, is varied (*9*). This problem is a major hurdle to applying flow reaction screening to medicinal chemistry, where limited material does not support preparation of multiple stock solutions. As an alternative, we envisioned preparing concentrated reactant and reagent solutions in a suitable solvent for each reaction component. These would be injected into a carrier solvent with the intervals between injections being carefully monitored to prevent cross-contamination between the individual reaction segments (*10*). Diffusion of the injected segment (5 µl) into the carrier solvent (500 µl, 1:100) would result in sufficient dilution for evaluation of the carrier solvent as the reaction solvent (*11*, *12*). The diluted reaction segments would continuously flow through a reactor coil with precise control of flow rate, temperature, pressure, and residence time. Real-time analysis of the segments as they emerged from the reactor coil, via fractionation into UPLC-MS, would eliminate subsequent off-line analysis time.

Flow technology system configuration

Our system configuration is depicted in Fig. 1A. Given the potential air and moisture sensitivity of the chemistries under evaluation and that we would be handling the ligands and catalysts in solution, the reactor was assembled in a glovebox environment.

The system uses a modified high-performance liquid chromatography (HPLC) system with a well-plate autosampler to prepare the reaction segments following a user-defined injector program, which guides the accurate aspiration and injection of microliter volumes from up to 192 source vials. Through this methodology, we optimized the system such that it takes the autosampler 45 s to assemble a reaction segment from, in this instance, five components composed of reactants 1 and 2 (for example, aryl halide and aryl boronic

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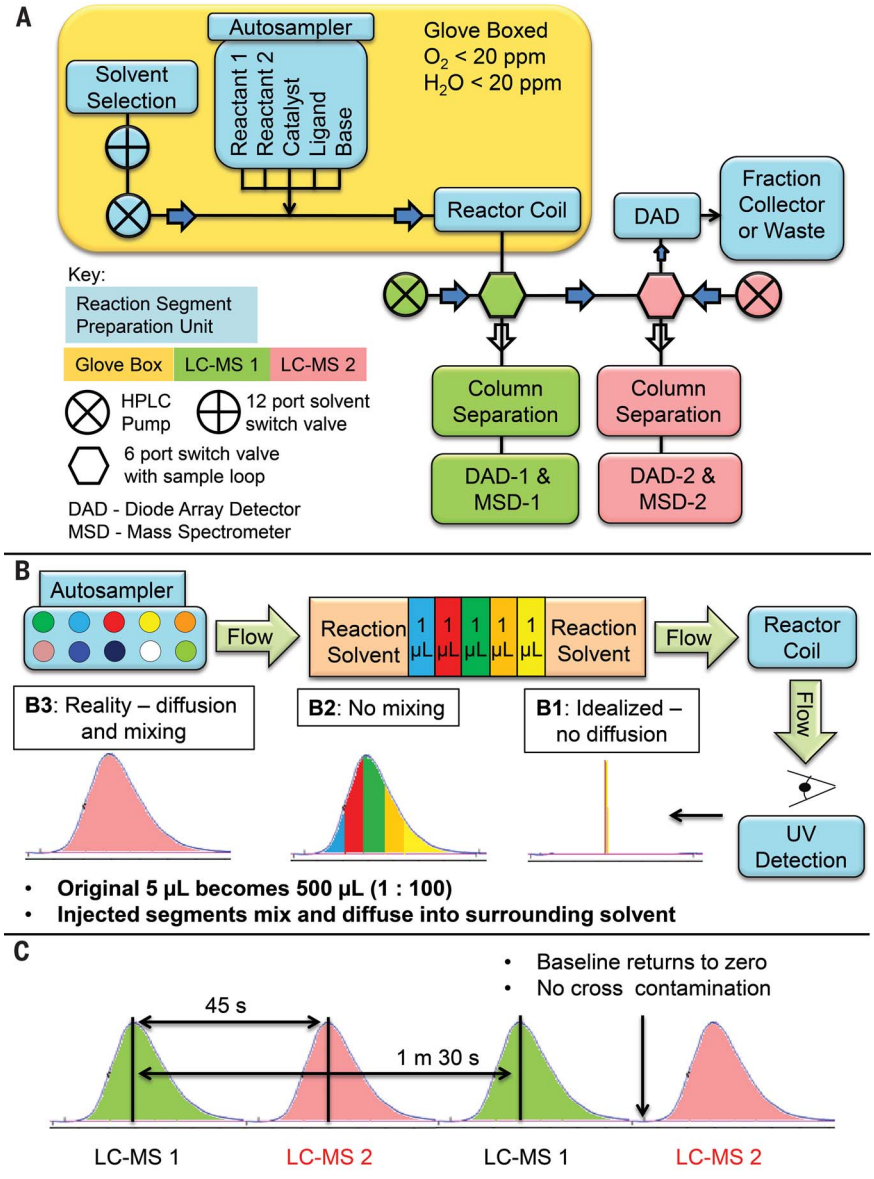

Fig. 1. Flow system setup and segment preparation. (**A**) Schematic depiction of the flow system. (**B**) Segment preparation and injection into flow stream showing potential mixing and diffusion outcomes, (B1) idealized–no diffusion, (B2) no mixing, and (B3) observed. (**C**) Portrayal of UV trace of the emerging reactions segments and fractionation into alternating LC-MS units.

acid in the case of a Suzuki-Miyaura coupling), catalyst, ligand, and base. The reaction segments are then injected into a flowing solvent stream (Fig. 1B), which is predetermined by the programmed method using the 12-port solvent selection value. The segment residence time in the temperature controlled reactor coil is determined by the pump flow rate.

To ensure maximal time efficiency, it is critical for the reaction samples to be analyzed as soon as they emerge from the reactor (*13*). To achieve this aim, two Agilent 1200 UPLC-MS instruments were positioned after the reactor outside of the glovebox so that while one was analyzing a reaction segment, the other was waiting to be triggered to analyze the next emerging segment (generated in 45-s intervals). In this manner, the three key dynamic components of the instrument (the two UPLC-MS instruments and the reaction segment preparation unit) are continuously working synergistically for maximum throughput. As a segment emerges from the reactor coil, it encounters a six-port switching valve, which directs it to the vacant LC-MS instrument for a detailed analysis (for clarity, segment 1 to LC-MS 1, segment 2 to LC-MS 2, etc.; Fig. 1C). The excess sample is directed through a diode array to enable visualization of the segment, and then either to a fraction collector or to waste, depending on the instrument's mode of operation.

Validation of four-component mixing in a model Suzuki-Miyaura reaction

With the general reactor design in hand, we first needed to assess the homogeneity of mixing of the various reaction components within the reaction segment, as well as the extent of diffusion of the segment itself into the surrounding carrier solvent, which is the key differentiating principle of this technology (*14*). Given its prevalent use in medicinal chemistry (*15*, *16*) as well as the extensive variables for optimization (ligands, Pd source, base), the Suzuki-Miyaura coupling between 6-bromoquinoline (**1b**) and the indazole boronic acid (**2a**) was chosen to validate the platform (Fig. 2A) (*17*). One of the challenges in evaluating homogeneity of mixing is that each of the reaction components will respond differently in the LC-MS, and the output trace will further be complicated by the extent of conversion to product. To counter this issue, we chose to make up the reaction component stock solutions of our model reaction (Fig. 2B) with inert internal standards such that the level of four of the five components could be accurately monitored. The internal standard used for each component was matched to the solubility, ensuring homogeneity in all cases. For the readily soluble components, ultraviolet (UV) active nonpolar solvents were used: **1b** dissolved in 1,3-diethylbenzene, $Pd(OAc)_2$ dissolved in 1,3,5-triethylbenzene, and PPh_3 dissolved in toluene. For **2a**, which is considerably less soluble, dimethylformamide (DMF) was used with 4,4′-di-*tert*-butylbiphenyl added as an internal standard. The fifth component, 1 M aqueous NaOH, was not directly monitored because of the difficulty in identifying an aqueous soluble inert internal standard. The molarities of the solutions were made such that a 1-µl addition of each (5 µl total) equates to a ratio of 1: 1: 2.5: 0.125: 0.0625 (**1a**: **2b**: base: ligand: Pd). With methanol as the carrier solvent, the segments were run with a flow rate of 1 ml/min through a Hastelloy coil heated to 100°C with a residence time of 1 min.

In this experiment, reaction segments of increasing volume were created with 1, 2, 4, 8, and 16 µl of each component (five components thus totaling 5, 10, 20, 40, and 80 µl) to assess the degree of mixing, the extent of diffusion, and the scalability of the reaction technology across a wide range of reaction volumes. To make up the segment, the five components were aspirated in series and then allowed to diffuse together, thus presenting a key differentiation with typical segmented flow systems in which diffusion is inhibited through use of a spacer, typically an inert gas or a perfluorinated solvent (*11*). Once the segments passed through the reactor coil, the output was split into a 96-well plate in 40-µl fractions. Each of the fractions was then analyzed via an off-line LC-MS by a standard method that enabled separation of each of the four internal standards mentioned previously. As shown in Fig. 2B, the ratio of each of the internal standards is approximately equivalent throughout the segment, demonstrating homogeneous diffusion of the reaction components into the

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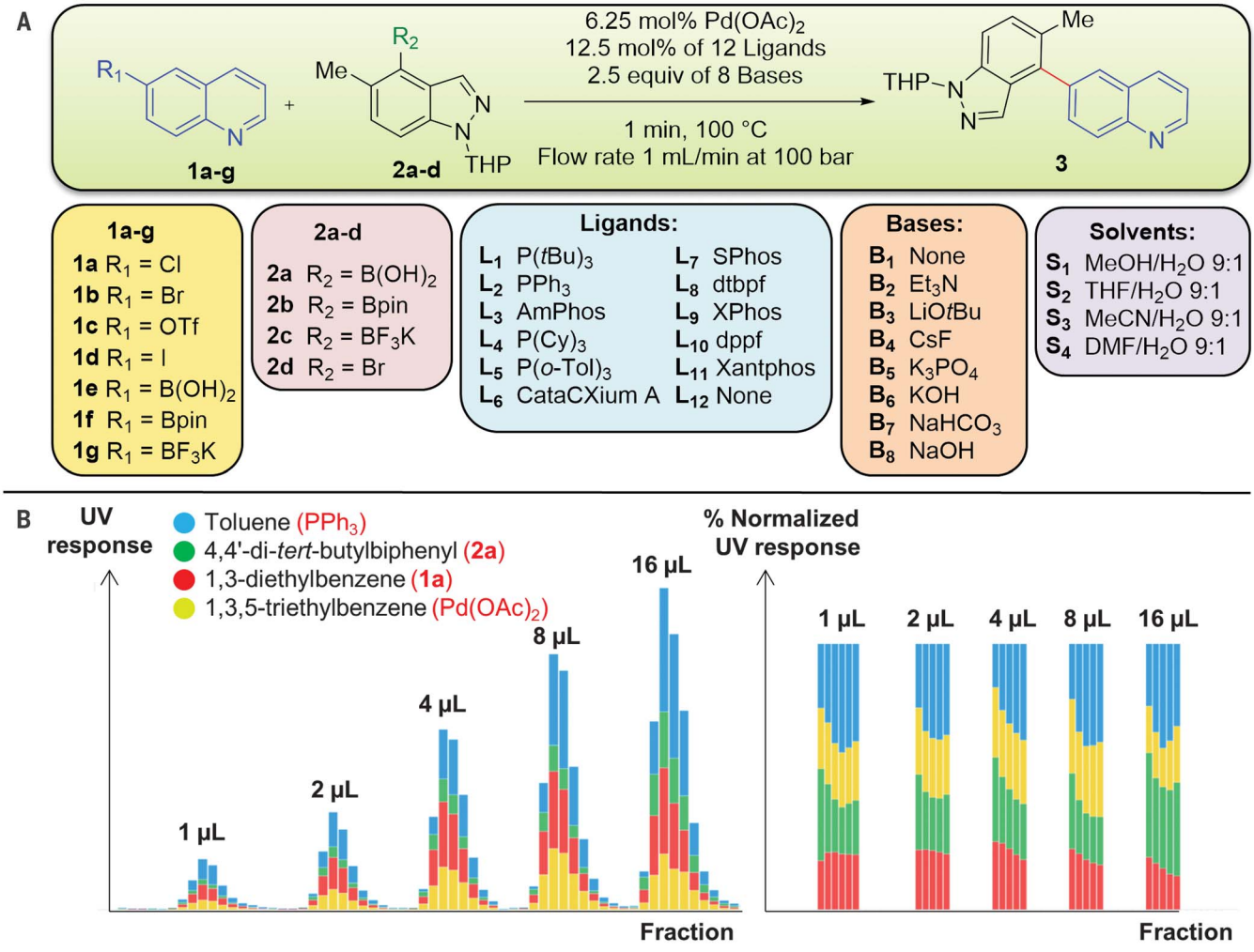

Fig. 2. Model Suzuki-Miyaura cross-coupling. (**A**) Coupling evaluates electrophiles **1a–1d**/**2d**, with **2a–2c**/**1e–1g** evaluated across a matrix of 11 ligands (plus one blank) × 7 bases (plus one blank) × 4 solvents. (**B**) UV analysis of internal standards (see legend) in the fractionated reaction segment derived from increasing volumes of components confirming adequate mixing.

surrounding carrier solvent, over a wide range of injection volumes. This result confirms that consistent reaction component stoichiometries can be generated throughout the segment and that larger segments can be injected to directly scale up screening results to produce meaningful quantities of material for biological evaluation. Additionally, these results show substantial homogeneous diffusion into the carrier solvent, which allows solvents to be screened with the same stock solutions in a continuous manner, a strategy that has not previously been achieved in the flow chemistry paradigm.

Rapid material-sparing screening of 5760 reactions

Judicious selection of both coupling partners plays a critical role, in terms of both the reactivity and economics of a Suzuki-Miyaura coupling (*18*). We therefore expanded upon our model transformation to execute on this concept, evaluating the coupling of electrophiles **1a** to **1d** with the nucleophiles **2a** to **2c** as well as the reverse combination **2d** with **1e** to **1g** across a matrix of 11 ligands (plus one blank) × 7 bases (plus one blank) × 4 solvents (Fig. 2A). Selection of the ligands was based on a series of factors, specifically (i) performance in previous internal batch screens; (ii) an internal principal component analysis of ligand property space (*19*, *20*); (iii) coverage of the main ligand classes from the literature (*21*); and (iv) commercial availability. The bases selected span a range of both organic and inorganic commonly used in this transformation, and solvents were chosen to display a range of polarities and dipole moments and the presence or absence of hydrogen bond donors (*22*). All reactions used $Pd(OAc)_2$ as precatalyst. Given that most Suzuki-Miyaura reactions use water as a cosolvent to solubilize the inorganic base employed and promote the formation of the boronate complexes involved in the transmetallation (*23*), the pump was set to provide a 9:1 solvent/water ratio (*24*). Overall, screening the complete set of variables would provide data for a total of 5760 reactions.

One important goal in our initial experimentation was to confirm that differences in reactivity between various reactions are due to the bulk carrier solvent and not the solvent in which the reagent stock solutions were made. Given the common nature of the solvents used to make up the stock solutions, we can infer that if there were a change in the reactivity during the solvent screen, it would be attributable to the carrier solvent mediating the reaction given the 100:1 dilution factor.

All relevant stock solutions were prepared under an inert atmosphere with the system set up as described previously for the validation of mixing experiments with the two UPLC-MS systems operating in tandem for analysis. The screen was run by making up successive reaction segments at 45-s intervals with 1 µl of each component stock solution. The stock solutions were

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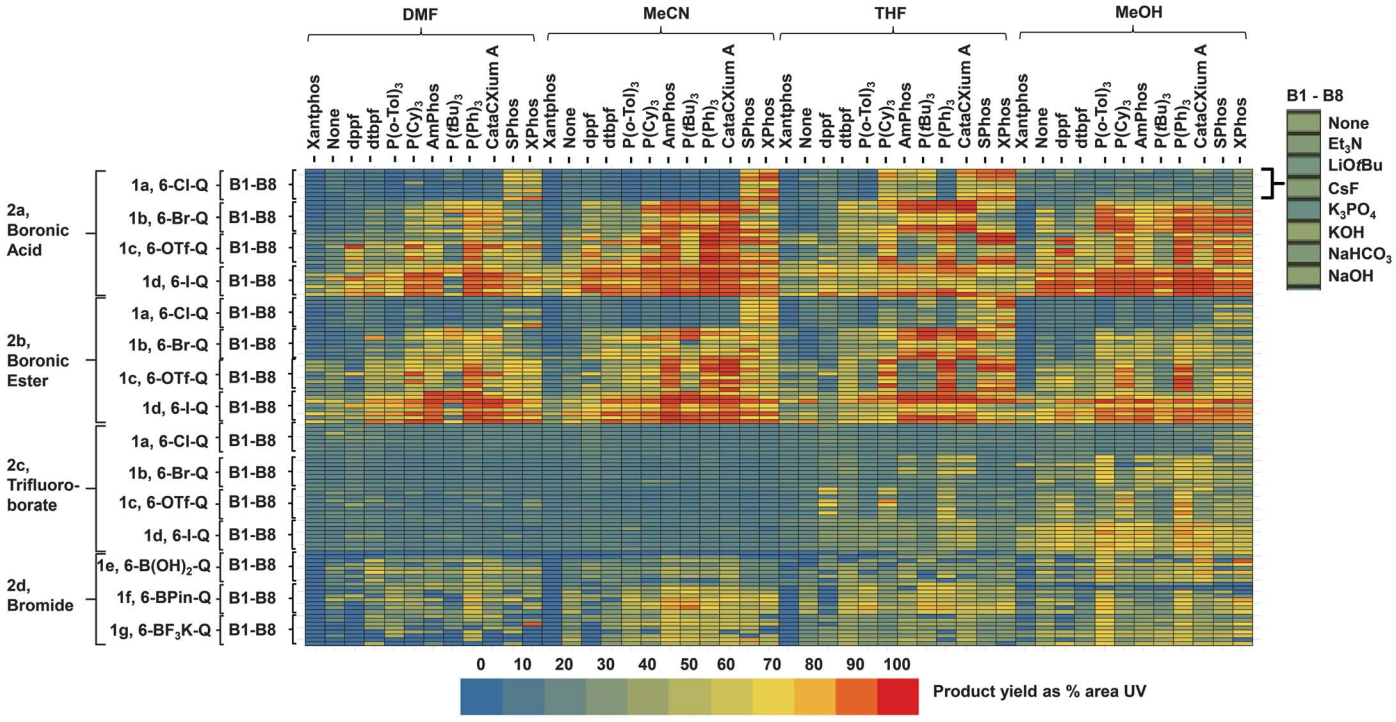

Fig. 3. Complete heatmap visualization. A total of 5760 reactions of **1a–1d** with **2a–2c**, and the reaction of **2d** with **1e–1g** evaluated across a matrix of 11 ligands (plus one blank) × 7 bases (plus one blank) × 4 solvents.

made up at molarity that equated to stoichiometries of 1: 1: 2.5: 0.125: 0.0625 (reactant 1: reactant 2: base: ligand: Pd). Each reaction was run with just 0.4 μmol, which in the case of **1a** equates to 65 μg each or 98 mg per 1500 reactions within 24 hours. The segments were then injected into the solvent stream flowing at 1 ml/min and 100 bar of pressure. This short reaction time is necessary to expedite throughput, and we anticipated that regardless of the absolute conversion to product, the relative conversions would allow the judicious selection of reaction conditions to be investigated for scale-up. Once a run was initiated, the system operated in a fully automated manner, at a rate of 1500 reactions per 24-hour time period. In contrast, a typical batch screen of 192 reactions in our laboratories runs for 18 hours (*25*).

To expedite data analysis, we used the Agilent Chemstation software in real time to identify key peaks in the LC-MS trace, followed by off-line refinement of the data through the iChemExplorer software before export and visualization with Spotfire, the latter allowing a large degree of flexibility in the identification of key reactivity trends. The overall process for data manipulation took approximately an hour for 1500 reactions. The complete set of data for the 5760 reactions is presented in the heatmap shown in Fig. 3.

Despite the large amount of data presented therein, it is possible to determine some high-level reactivity trends from this visualization. First, the 6-chloroquinoline electrophile **1a** is clearly the worst substrate, although there are conditions identified showing good reactivity (specifically with ligands such as XPhos and SPhos) (*26*, *27*). The relative reactivity of the quinoline cores can be more explicitly seen by the box plot shown in Fig. 4A, which compares the yields of these four electrophiles (**1a** to **1d**) in the reactions with both the boronic acid (**2a**), BPin (**2b**), and BF_3K (**2c**) derivatives. Furthermore, it is clear that a higher density of conditions provides high conversion when the indazole is used as the nucleophilic partner rather than as the electrophile.

Second, comparison of the boron sources shows that the BF_3K derivative **2c** is inferior to both the boronic acid **2a** and ester **2b**, which may reflect that the 1-min residence time is not sufficient for the BF_3K (**2c**) derivative to hydrolyze to the boronic acid (**2a**) for coupling (*23*). However, in the cases where the BF_3K (**2c**) has shown reactivity, the use of MeOH (or to a lesser degree tetrahydrofuran (THF) as the solvent is key (Fig. 4A), thus validating the concept of identifying solvent reactivity trends through variation of the bulk carrier solvent.

Focusing specifically on the reaction of the boronic acid **2a** with the four quinoline-based electrophiles **1a** to **1d**, again we can clearly see that chloroquinoline **1a** performs most poorly (Fig. 4B). However, it is also possible to determine further trends specifically for the other electrophiles. We can observe that in general, for bromoquinoline **1b**, methanol appears to be the superior solvent. We can also see that in general, Xantphos is a poor choice of ligand for the reaction, whereas PPh_3 can give high conversions to the desired product for all but the Cl-quinoline, particularly in MeOH and MeCN (*28*).

Finally, it is possible to filter the conditions, and cluster by those that provide a conversion of greater than 85% for all of the electrophiles screened. This result, shown in Fig. 5, demonstrates that from the 384 potential conditions evaluated, three will work well independent of the selection of the electrophile, and these are all based either on X-Phos or S-Phos and use MeCN as the solvent. This finding is key for identifying conditions that are most amenable for parallel synthesis—for example, wherein one seeks reactivity across the broadest substrate scope.

To demonstrate the capability of the instrument for providing milligram quantities of material through the processing of multiple segments, we chose a representative set of reaction conditions from the screening results discussed. Using these conditions, we programmed the autosampler to inject 100 consecutive segments consisting of 8 μl per reaction component (3.2 μmol per segment), which would be able to provide a maximum of 110 mg of desired material in ~75 min. The combined segments were collected and evaporated, and the product isolated in 59% yield (65 mg) after purification by column chromatography (Fig. 6A). The reaction conditions identified were translated to further scale-up in continuous flow with a commercially available Vaportec R series reactor, with 42% of the desired product being obtained by means of the standard two-pump setup (*29*).

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We next aimed to demonstrate reproducibility and direct translation to traditional batch reaction operations. With no further optimization or experimentation in batch, the flow-identified conditions were altered prospectively only to make the overall reaction more concentrated (0.10 M) and carried out with typical Schlenk line techniques (*30*). Gratifyingly, the reaction performed well in batch and gave an isolated yield of 79% for the desired coupling product, on time scales similar to that for the flow optimization work (Fig. 6A). To further validate the results obtained from the screen, several additional permutations of reaction conditions including solvents, ligands, and coupling partners were run in a batch manner. Two positive controls gave isolated yields of 67 and 93%, respectively, whereas two negative controls gave either <10% conversion or no trace of product after reaction overnight (see supplementary materials for detailed experimental conditions for control scale-up experiments 9 to 12). Although these further results cannot totally rule out the possibility of "false positives" or "false negatives" in the data set, they build further confidence in the validity and reproducibility of the experimental outcomes provided by this method.

To further demonstrate the utility of the reaction platform for optimizing a particularly challenging Suzuki-Miyaura coupling, we did an analysis of recently conducted Suzuki-Miyaura coupling parallel synthesis libraries [parallel medicinal chemistry (PMC)] within ongoing Pfizer discovery projects. The bromo-oxindole, **5**, rose to the top as an electrophile that was present in the design of 13 libraries (*31*), reflecting its desirable properties from a medicinal chemistry perspective, but failed to provide the desired product in all cases. A screen of conditions, which at 0.4-μmol scale per reaction equates to 50 mg of **5** per 576 reactions in 8 hours for the coupling with boronic ester **4**, showed a stark disparity in the efficiency of conditions for this coupling (Fig. 6B), with only a few providing moderate (45 to 65%) conversion to the desired product during the 1-min reaction time screened, as indicated on the heatmap (Fig. 6C). These results suggested that CataCXium A (*32*) was a uniquely effective catalyst with THF/H_2O as the preferred solvent and MeOH/H_2O as a potential alternative (*33*). Et_3N was preferred as the base, with NaOH, CsF, and K_3PO_4 emerging as possible inorganic alternatives. Finally, the conditions typically used for our PMC synthesis [$Pd(OAc)_2$, $P(Cy)_3$, THF/H_2O] completely failed in the flow screen, thus supporting the original findings.

Direct translation of these optimal conditions to batch, with the exception of reaction concentration and elongated reaction time, afforded high conversion and isolated yield of the coupling product, which was not observed in the PMC efforts (Fig. 6B).

Outlook

The platform described in this study provides a notable advance in the ability to screen reaction reagents, solvents, and conditions in a flow-based

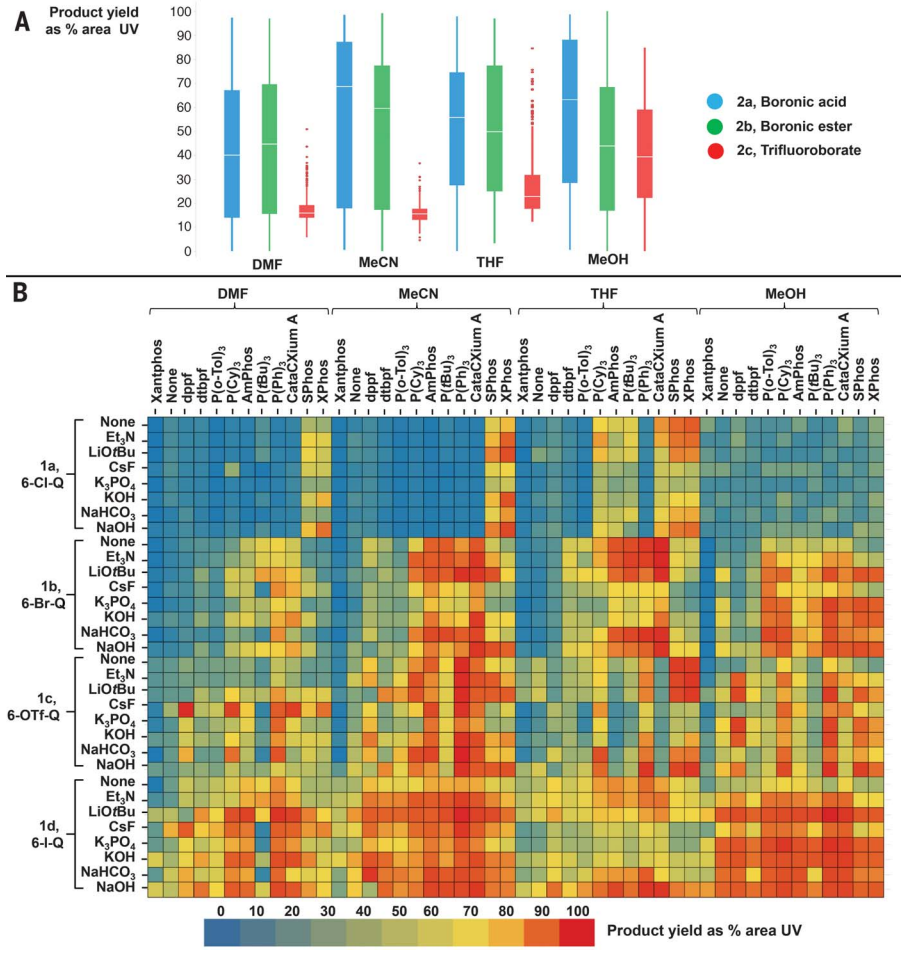
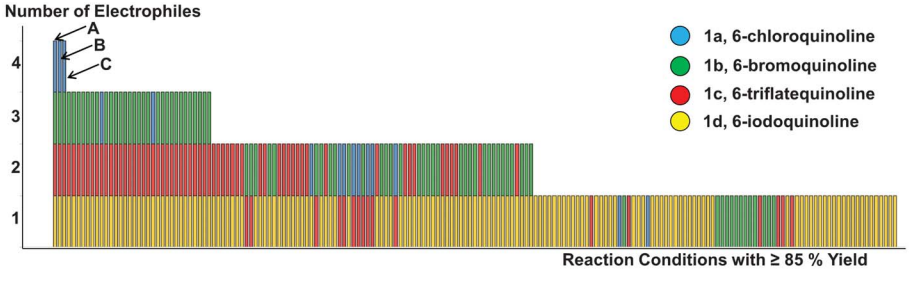


Fig. 4. Data analysis of model Suzuki-Miyaura cross-coupling. (**A**) Box plot comparison of quinoline electrophiles **1a–1d** in reactions with boronic acid **2a** and boronic ester derivatives, **2b** and **2c**, across different solvents. (**B**) Heatmap of coupling of quinoline electrophiles **1a–1d** with **2a** evaluated across a matrix of 11 ligands (plus one blank) × 7 bases (plus one blank) × 4 solvents.



Reaction Condition	Solvent	Base	Ligand	6-Cl-Q (1a)	6-Br-Q (1b)	6-OTf-Q (1c)	6-I-Q (1d)
A	MeCN	LiO*t*Bu	SPhos	85	92	91	93
B	MeCN	NaOH	SPhos	86	93	91	88
C	MeCN	NaOH	XPhos	93	90	92	86

Fig. 5. Suzuki-Miyaura cross-coupling—successful conditions. Conditions with ≥85% yield for all quinoline-based electrophiles **1a–1d** with **2a**. This indicates that 181 conditions work for one electrophile, 103 conditions work for two electrophiles, and 33 conditions work for three electrophiles, whereas only 3 sets of reaction conditions (specified in the table) work for all four electrophiles.

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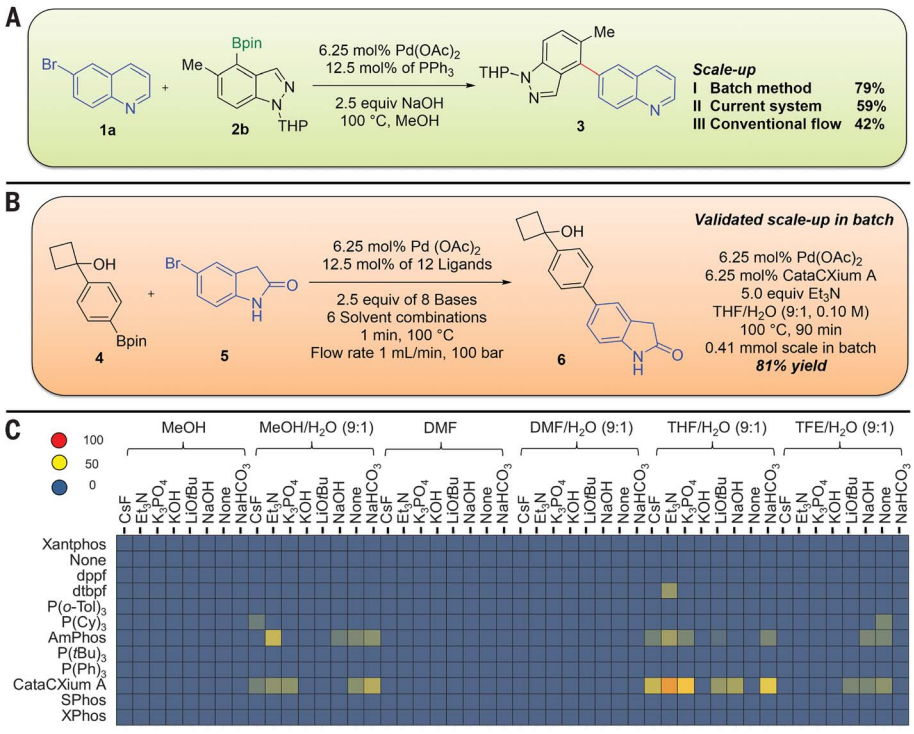

Fig. 6. Scale-up validation and PMC optimization. (**A**) Scale-up validation of reaction conditions in both batch and flow for the Suzuki-Miyaura cross-coupling of **1b** with **2b**. (**B**) PMC optimization via flow-screening and validation in batch for the coupling of **4** with **5** evaluating a matrix of 11 ligands (plus one blank) × 7 bases (plus one blank) × 6 solvents. (**C**) Complete heatmap visualization of the 576 reactions.

manner. Although it is typically difficult to directly compare batch and flow platforms based on the limited information provided regarding setup, the ability described here to run more than 1500 reactions in a 24-hour period with high-resolution information-rich reaction analysis benchmarks this methodology favorably against plate-based techniques. In addition, there are a number of advantages, beyond the generation of real-time analytical data, associated with running the reactions in a flow paradigm, including avoiding solvent evaporation, improved mixing, and uniform heating. The system at present is geared toward the analysis of homogeneous reactions, and thus issues arising from inefficient mixing of either heterogeneous or biphasic reaction systems present a gap, which remains to be addressed in the current technology.

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30. A potential question arises as to whether the solvent used to make up the stock solution can influence the reaction. However, as demonstrated by Jouyban *et al.* (*36*), the impact of a mixed solvent in terms of a dielectric constant is a weighted average of the mixed components. As such, the effect of injecting a different solvent into a 9/1 organic/aqueous mixture that is diluted out 1:100 will largely be negated so long as it is inert to the chemistry being screened. The validity of this argument is borne out by the fact that the scaled batch experiments work in a similar manner to the flow experiments even without the solvents used to make up the stock solutions. However, although no effect of the small amount of stock solvent present in the reaction system has been observed thus far, we cannot preclude its potential involvement in all transformation screening.
31. The monomer **5** was used in 13 in-house library campaigns all involving Pd-mediated couplings (9 were Suzuki-Miyaura couplings whereas the remainder were Buchwald-Hartwig reactions). In none of the examples evaluated did **5** lead to any of the desired product in the library matrix.
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33. These results reinforce the efficiency of the mixing within the system, including the aqueous component. In this experiment, two systems are evaluated in which "pure" organic solvents (DMF, MeOH) have been utilized, with no reactions performing well. This is to be expected as the experiment uses the BPin derivative, which to react requires hydrolysis to the $B(OH)_2$ derivative (*22*). If sufficient mixing did not occur with the aqueous-based systems then poor reactivity would be expected for all reactions.
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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/359/6374/429/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S24
Tables S1 to S3
References (*37*–*40*)
Data File S1

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A platform for automated nanomole-scale reaction screening and micromole-scale synthesis in flow

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A reaction screen in flowing solvent

Chemists charged with manufacturing pharmaceuticals have recently been exploring the efficiency advantages of continuous flow techniques. Perera *et al.* now show that a flow apparatus can also accelerate reaction optimization earlier in the drug discovery process. They modified a high-performance liquid chromatography system to screen a wide variety of solvent, ligand, and base combinations to optimize carbon-carbon bond formation. Injecting stock solution aliquots of the catalyst and reactants into a carrier solvent stream let the authors vary the main solvent efficiently and scale up the optimal conditions for product isolation.

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