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Research Article

High-Throughput Experimentation and Continuous Flow Evaluation of Nucleophilic Aromatic Substitution Reactions

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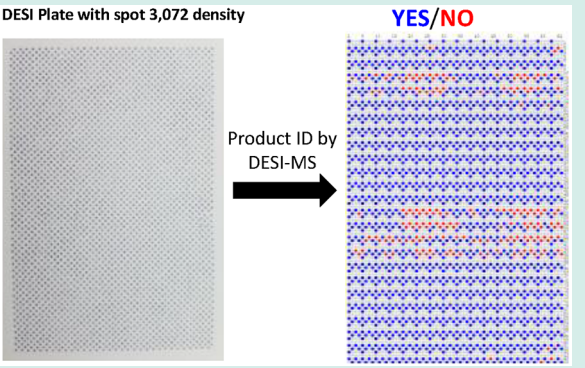
ABSTRACT: Nucleophilic aromatic substitution (S_NAr) reactions were optimized using high-throughput experimentation techniques for execution under flow conditions. A total of 3072 unique reactions were evaluated with an analysis time of ∼3.5 s per reaction using a system that combines a liquid handling robot for reaction mixture preparation with desorption electrospray ionization (DESI) mass spectrometry (MS) for analysis. The reactions were performed in bulk microtiter arrays with and without incubation. In-house developed software was used to process the data and generate heat maps of the results. This information was then used to select the most promising conditions for continuous synthesis under microfluidic reactor conditions. Our results show that this HTE approach provides robust guidance for narrowing the range of conditions needed for optimization of S_NAr reactions.



KEYWORDS: *high-throughput experimentation, nucleophilic aromatic substitution, flow chemistry, desorption electrospray ionization mass spectrometry, DESI-MS*

INTRODUCTION

High-throughput experimentation (HTE) allows for the implementation of large numbers of experiments in parallel, requiring less labor per experiment,[1] and utilizing small amounts (picogram−nanogram) of material. This technique can boost lab productivity by rapid generation of comprehensive data for the selected transformation.[1,2] HTE-based experiments focused across a range of variables have spread in biology, drug discovery,[3] medicinal chemistry,[4,5] and catalysis.[6,7] Analysis of the resulting large data sets to extract a deeper understanding of the chemical transformation, however, can be a bottleneck in the discovery process. The identification and optimization of reaction conditions prior to chemical process development can be accelerated when HTE is coupled with MS analysis.[6,8,9] These impacts are particularly evident in the pharmaceutical and biopharmaceutical industries where reduction in the time required to execute each experimental cycle is a necessity due to the high value of these product classes.[10,11] The HTE method reported herein are based on two techniques to identify promising reaction conditions for scale up: (i) desorption electrospray ionization mass spectrometry (DESI-MS) and (ii) bulk microtiter (small scale batch) reactions.

DESI-MS is an ambient ionization technique,[12] wherein electrospray droplets are directed onto a surface generating a thin film of solvent (∼300 μm in diameter). Analytes present on the surface desorb from this thin film and are then transferred into the mass spectrometer by secondary droplets that are generated from small splashes of continuously incoming electrospray droplets. The DESI-MS inlet is then rastered over the surface using a moving $x-y$ stage to generate a 2D map of chemical information in the form of full mass spectra. The thin films and droplets generated in the DESI-MS process can lead to reaction acceleration.[13] This has been demonstrated previously with amine alkylation,[9,14] Suzuki coupling,[15] and aldol reactions.[16] In this study, DESI-MS was also used to analyze the results of the bulk microtiter reactions run at elevated temperatures that are not easily achieved in the DESI-MS format. Because the analysis occurs at a rate of ∼3.5 s per reaction mixture, DESI enables a far more rapid evaluation of reaction outcomes than other techniques, such as LC-MS, that typically take several minutes for each sample. It is important to note that we will refer to the droplet/thin film reactions as desorption electrospray ionization (DESI) reactions in this Research Article. This is not to be confused with the DESI-MS analysis of the bulk microtiter reactions.

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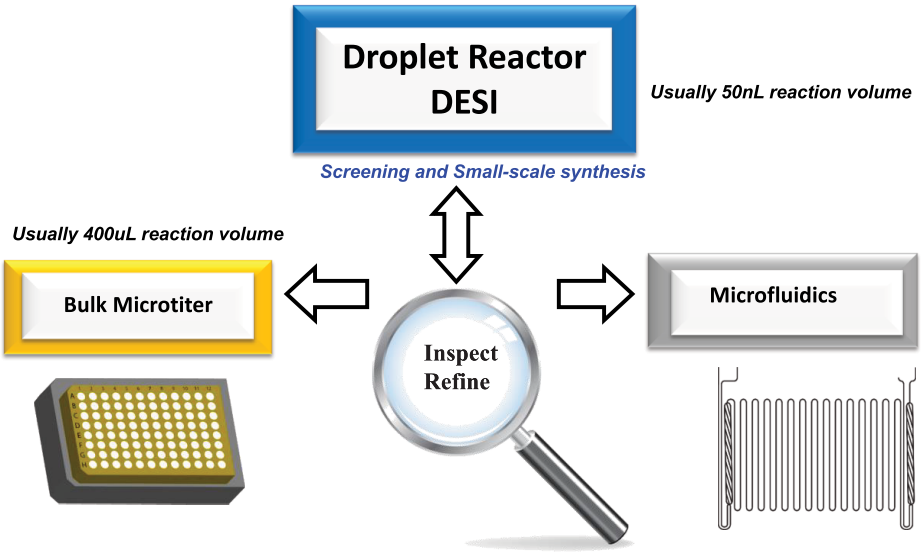
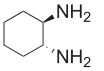
Scheme 1. Reaction Formats Employed in This Study



Scheme 2. Reagents Used in Round 1 of the HTE Campaign[a]

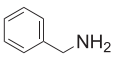
Amines


R1-A1
Exact Mass: 114.12


R1-A2
Exact Mass: 82.05


R1-A3
Exact Mass: 85.09


R1-A4
Exact Mass: 100.10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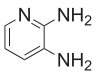
Exact Mass: 107.07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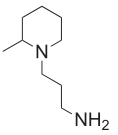
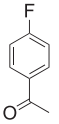
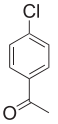
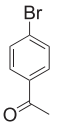

R1-A7
Exact Mass: 156.16

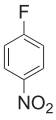

R1-A8
Exact Mass: 118.05

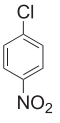
Aryl halides

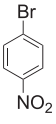

R1-B1
Exact Mass: 138.05

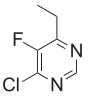

R1-B2
Exact Mass: 154.02

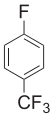

R1-B3
Exact Mass: 197.97

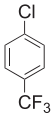

R1-B4
Exact Mass: 141.02

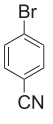

R1-B5
Exact Mass: 156.99

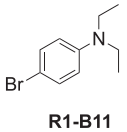

R1-B6
Exact Mass: 200.94

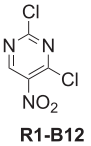

R1-B7
Exact Mass: 160.02


R1-B8
Exact Mass: 164.02


R1-B9
Exact Mass: 180.00


R1-B10
Exact Mass: 180.95


R1-B11
Exact Mass: 227.03


R1-B12
Exact Mass: 192.94

[a]R1 refers to round 1 of the HTE test. A# refers to the amine type, while B# refers to the electrophile type.

Nucleophilic aromatic substitution (S_NAr) reactions are versatile transformations in the organic chemistry arsenal,[17] and one of the important reactions used for making pharmacologically[18,19] and biologically active molecules.[20−25]

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Table 1. Direct Comparison of S_NAr Reactions Using Droplet/Thin Film and Microtiter Approaches[a]

A)

	R1-B1	R1-B2	R1-B3	R1-B4	R1-B5	R1-B6	R1-B7	R1-B8	R1-B9	R1-B10	R1-B11	R1-B12 (S)	B12 (D)
R1-A1													
R1-A2													
R1-A3													
R1-A4													
R1-A5													
R1-A6													
R1-A7													
R1-A8													

B)

	R1-B1	R1-B2	R1-B3	R1-B4	R1-B5	R1-B6	R1-B7	R1-B8	R1-B9	R1-B10	R1-B11	R1-B12 (S)	B12 (D)
R1-A1													
R1-A2													
R1-A3													
R1-A4													
R1-A5													
R1-A6													
R1-A7													
R1-A8													

C)

	R1-B1	R1-B2	R1-B3	R1-B4	R1-B5	R1-B6	R1-B7	R1-B8	R1-B9	R1-B10	R1-B11	R1-B12 (S)	B12 (D)
R1-A1													
R1-A2													
R1-A3													
R1-A4													
R1-A5													
R1-A6													
R1-A7													
R1-A8													

D)

	R1-B1	R1-B2	R1-B3	R1-B4	R1-B5	R1-B6	R1-B7	R1-B8	R1-B9	R1-B10	R1-B11	R1-B12 (S)	B12 (D)
R1-A1													
R1-A2													
R1-A3													
R1-A4													
R1-A5													
R1-A6													
R1-A7													
R1-A8													

[a](A) The droplet/thin film and (B) bulk microtiter results for the same set of reaction conditions. Experimental conditions: DESI solvent, methanol; reaction solvent, NMP; base, DIPEA. (C) Same as panel B, but the DESI spray solvent was MeOH+ 1% FA. (D) Same as panel C, but the reaction solvent was 1,4-dioxane. Each cell is an average of two data points. Green cells represent "yes" reactions (product ion intensity ≥ 150 counts). Red cells represent "no" reactions (product intensity < 150 counts). B12 can form both single and double addition products; the double addition product can form multiple ions. B12(S) is the singly charged ion of the single addition product, while B12(D) is the sum of the average intensities of all the double addition ions.

The reaction mechanism of this transformation[26,27] involves a stepwise addition−elimination sequence,[28−30] wherein the first step involves a nucleophilic attack of the substrate to provide a Meisenheimer complex, followed by the loss of the leaving group through either catalyzed or noncatalyzed pathways.[30−32] The reaction typically involves an amine as the nucleophile,[26] although a wide variety of non-nitrogenous nucleophiles may be used. This study reports HTE evaluation of S_NAr reactions performed in both droplets/thin film and bulk microtiter formats with analysis by DESI-MS. After HTE evaluation, validation of the reaction hotspots were performed in flow to increase confidence in the HTE findings (Scheme 1).

Microfluidic reactions are attractive alternatives for organic synthesis, since continuous flow methods have shown great potential to achieve faster and greener transformations,[33] including the microfluidic synthesis of active pharmaceutical ingredients using ESI-MS analysis.[34−36] Although the S_NAr reaction is already known in flow,[37] we selected a broader range of substrates for this study in an effort to develop efficient flow-enabled routes to biologically as well as

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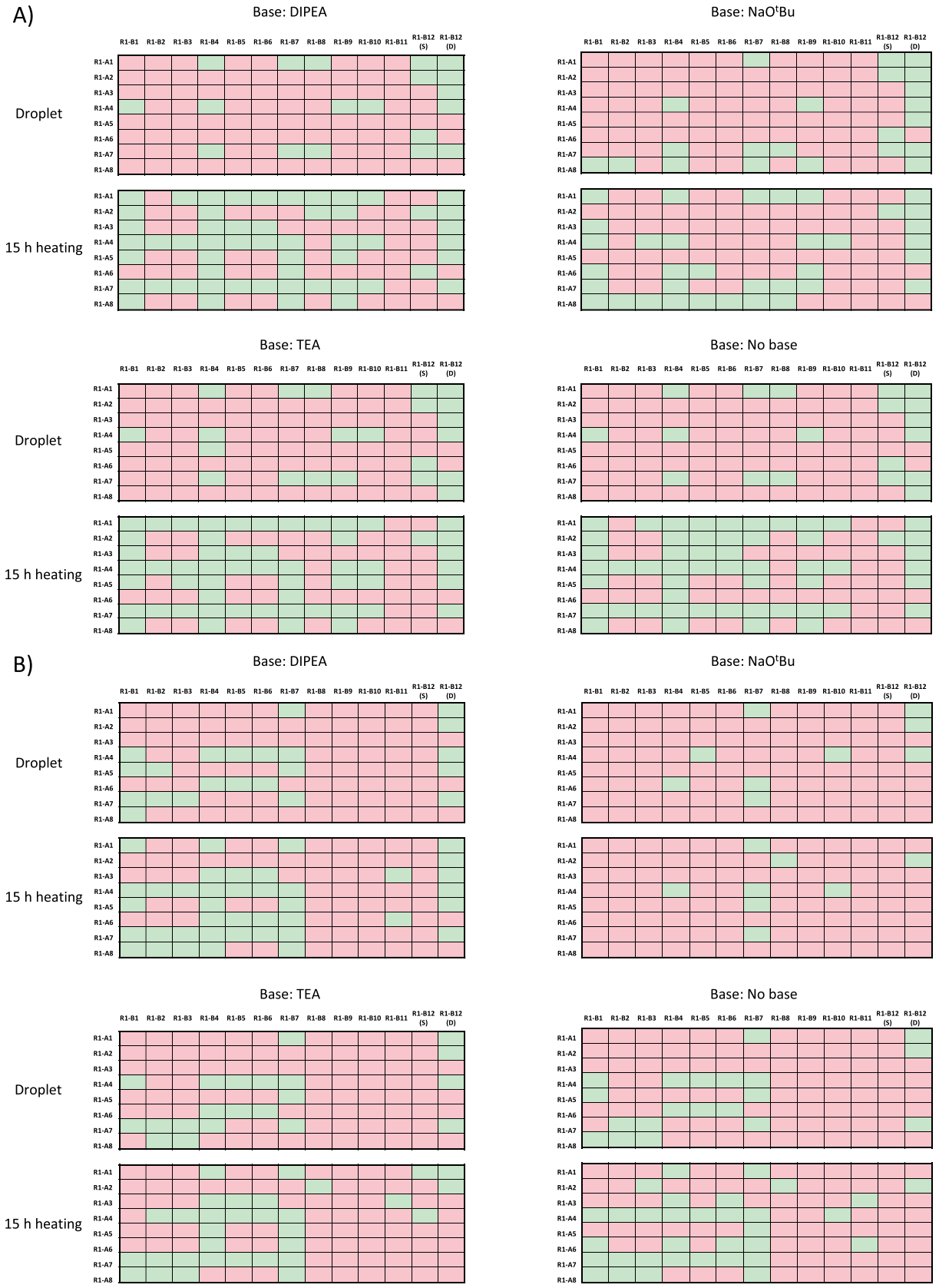

Figure 1. Heat map of 1536 reactions from round 1 of the S_NAr HTE using MeOH with 1% FA as DESI spray solvent under droplet/thin film or bulk microtiter plate conditions at 150 °C for 15 h using four different bases. (A) Reaction solvent, NMP; (B) reaction solvent, 1,4-dioxane. Green cells represent successful reactions (average product intensity ≥ 150 counts). Red cells represent unsuccessful reactions (average product intensity < 150 counts).

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pharmaceutically important synthons.[15] The preparation of S_NAr reaction mixtures for both the DESI and microtiter HTE methods was performed in glass-lined 96-well metal plates using 16 different amines and 13 different aryl halides. Additional variables, including base, reaction solvent, DESI spray solvent, temperature, and reaction time, were also evaluated.

RESULTS AND DISCUSSION

High-Throughput Experimentation (HTE). Two high-throughput experimentation methods were tested for reaction evaluation: DESI at room temperature (transformations in droplets/thin films, used for both synthesis and analysis) and bulk microtiter plates at elevated temperature (DESI for analysis only). *N*-Methyl-2-pyrrolidone (NMP) and 1,4-dioxane were chosen as the polar aprotic solvents for these reaction because all the reagents dissolved in these solvents.[38] DESI-MS analysis allowed rapid investigation of reactions that were capable of producing a diverse product profile. The spray solvents were MeOH or MeOH with 1% formic acid (FA). Full mass spectra in positive mode were recorded for each reaction mixture.

Eight amines and 12 aryl halides (Scheme 2) were tested in the first experiment using different bases in NMP and 1,4-dioxane. The bulk microtiter HTE reactions were heated for S_NAr reaction product formation at 150 °C for 15 h.

The amine and aryl halide ratios used were 1:1, and the different bases were used at 2.5 equiv relative to aryl halide. A Beckman-Coulter Biomek i7 liquid handling robot was used to prepare the reaction mixtures in either 96-well glass-lined metal blocks or 384-well plates. A total of 400 μL of the reaction solution was prepared in each well of the 96-well plates. Four identical 96-well plates were prepared, each utilizing one of four different base conditions: *N*,*N*-Diisopropylethylamine (DIPEA), sodium *t*-butoxide (NaO^tBu), triethylamine (TEA), and no base (control). Each reaction mixture (40 μL) was then transferred to a 384-well plate (i.e., aliquots from all four 96-well glass-lined metal plates deposited into one 384-well plate), followed by transfer of 50 nL of each reaction mixture in the 384-well plate to a PTFE surface (a porous polytetrafluoroethylene sheet glued onto a glass support) using a 384-format stainless steel pin tool. This final transfer is necessary because the reaction mixtures must be on a planar surface to enable DESI-MS analysis.

Up to 16 384-well plates can be pinned onto one PTFE surface (also referred to as the DESI slide) by slightly offsetting the location of the pins relative to the surface during each transfer as described by Wleklinski et al.,[9] resulting in a total of up to 6144 spots per DESI slide, including a collection of rhodamine spots that serve as fiducial markers. It is important to note that these reaction mixtures were placed onto the surface prior to any incubation steps for evaluation of the droplet/thin film reactions. The remaining reaction solutions in the metal blocks were heated for 15 h at 150 °C to initiate the bulk reactions. After heating, the well plates were cooled, and samples of the reaction mixtures were transferred to the PTFE surface using the same procedure described above. These thermally activated bulk microtiter reaction mixtures were spotted onto the same PTFE surface as the nonincubated mixtures to enable direct comparison. The PTFE surface was then analyzed using DESI-MS and the MS data analyzed using in-house software called Chemical Reaction Integrated Screening (CHRIS) to produce heat maps of the reaction outcomes.[39]

We performed two rounds of HTE, differing primarily in the amine nucleophiles used. A subset of the data from round 1 is shown in Table 1. Each square in Table 1 represents a unique reaction condition using DIPEA as base and is an average of two separate reaction replicates. The peak intensities of the products in the corresponding full scan mass spectra were used to evaluate the success or failure of each reaction. A successful reaction was defined as having a product peak intensity of at least 150 counts ($S/N \sim 5$) in the centroided mass spectrum.

Twelve successful reactions were found for the droplet reactions, whereas 41 successful reactions were found for the bulk microtiter transformations (Table 1A and B). There was no doubt that some of the reactions were favorable under droplet conditions, however, S_NAr reactions typically require heating,[40] so it was not surprising that more "yes" reactions were observed under the heated bulk reaction conditions employed. Moreover, MeOH with 1% FA was found to be a better spray solvent than MeOH due to better product ionization in the presence of acid[41,42] (Table 1C). This change increased the number of successful reactions detected by 30% (54 count). It is also worth noting that the reaction worked better in NMP than 1,4-dioxane (54 "yes" reactions vs 40 "yes" reactions) (Table 1C and D) since NMP is much more polar.[38] We attribute these finding to the stabilization of the sigma complex intermediate produced after the addition step.

Figure 1 shows the heat map of the round 1 reactions in both DESI and bulk microtiter format using MeOH with 1% FA as the spray solvent. In general, electron-donating groups (EDG) in the amine nucleophile and electron withdrawing groups (EWG) in the aryl halide substrate favored product formation.[26,27] For these experiments, the most reactive amines were 1-methylpiperazine (**R1-A4**) and 3-(2-methylpiperidin-yl)propan-1-amine (**R1-A7**), both of which possess electron-donating groups. Similarly, a strong electron-withdrawing nitro group in the electrophile (e.g., 1-fluoro-4-nitrobenzene (**R1-B4**), 1-chloro-4-nitrobenzene (**R1-B5**), 1-bromo-4-nitrobenzene (**R1-B6**), and 2,4-dichloro-5-nitropyrimidine (**R1-B12**)) increases the reaction extent for these aryl halides, whereas 4-bromo-*N*,*N*-diethylaniline (**R1-B11**) did not work well because of the diethyl amine electron donating group. All other aryl halides reacted to the same extent. Ortho substituents in the amine nucleophile or aryl halide substrate retarded the reaction due to steric hindrance.[43] Thus, pyridine-2,3-diamine, **R1-A6**, or 2,4-dichloro-5-nitropyrimidine (**R1-B12**) did not react well, although (1*R*,2*R*)-cyclohexane-1,2-diamine (**R1-A1**) reactions were facile since the two adjacent amino groups are on different faces of the cyclohexane ring. 1-Methyl-1*H*-imidazole (**R1-A2**) did not react (see Tables SI1–4 for the ion intensities observed in these experiments).

In spite of the structural similarities of piperidine (**R1-A3**) and 1-methylpiperazine (**R1-A4**), **R1-A4** always showed better reactivity than **R1-A3** because of the presence of an electron donating group (EDG) that enhances its nucleophilicity. Moreover, benzylamine (**R1-A5**), pyridine-2,3-diamine (**R1-A6**), and benzimidazole (**R1-A8**) showed lower reactivity due to the presence of an electron-withdrawing aromatic moiety in these molecules, making them less nucleophilic toward the addition step in the reaction mechanism.

The summary of successful S_NAr reactions detected upon analysis of 1536 unique droplet/thin film and bulk reactions is shown in Table 2. Among all the reactions, 311 "yes" reactions

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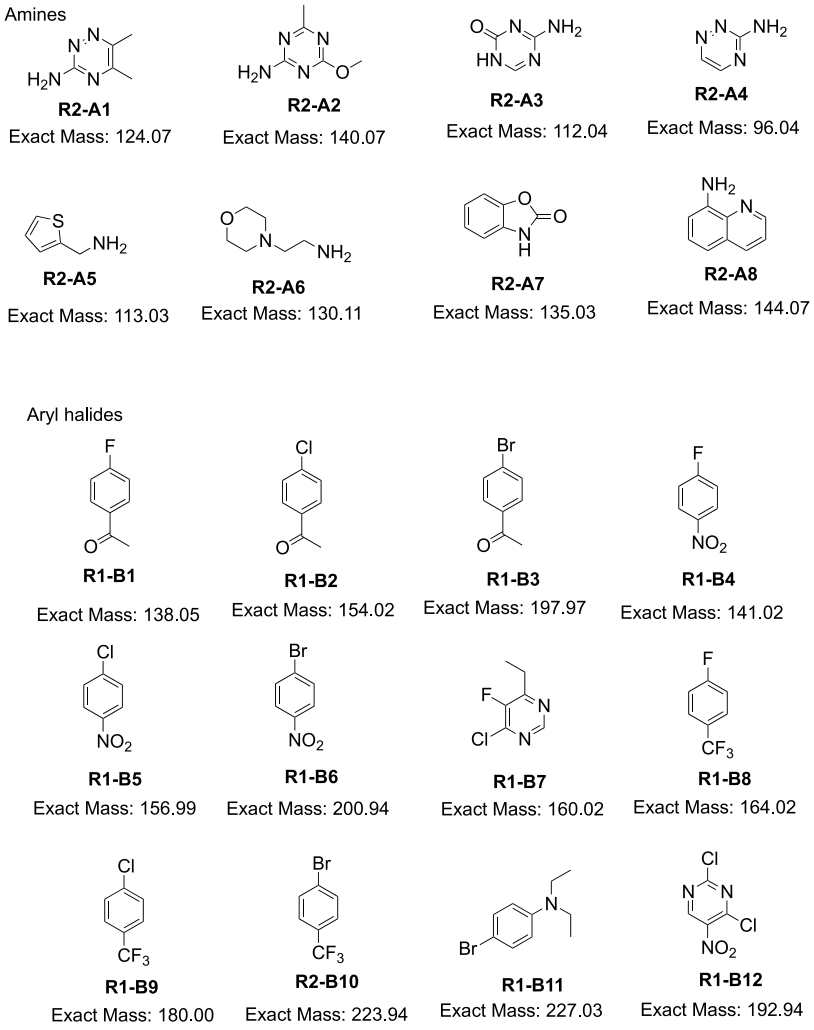
Table 2. S_NAr Product Formation Outcomes for 96 Reactions As a Function of Reaction Solvent, Base Type, and DESI-MS Spray Solvent

Number of successful reactions in droplet/thin film format								
Base →	DIPEA		NaO^{t}Bu		TEA		No Base	
Reaction solvent→ /Spray solvent ↓	NMP	Dioxane	NMP	Dioxane	NMP	Dioxane	NMP	Dioxane
MeOH	12	--	10	--	21	--	13	--
MeOH with 1% FA	18	22	22	09	22	21	19	20
Number of successful reactions in bulk microtiter format								
MeOH	41	--	18	--	40	--	41	--
MeOH with 1% FA	54	40	35	08	55	32	54	33

Scheme 3. Reagents Used in Round 2 of the HTE Campaign



were observed in bulk, while less than half that number were observed (153) when run in the droplet/thin film format.

Our initial HTE campaign was followed by a second round of S_NAr reactions using a family of biologically active amine synthons. Transformations in this round employed the same aryl halides (except that **R1-B10** was exchanged for 1-bromo-4-(trifluoromethyl)benzene, **R2-B10**) with a different set of eight amines (Scheme 3). The reaction conditions for round 2 were similar to round 1, except that (i) all reactions were performed in NMP, (ii) time points of 1, 4, and 15 h at 150 °C were used for the bulk mictotiter reactions, and (iii) methanol with 1% FA was the only DESI spray solvent used.

Figure 2 shows the heat maps from round 2. Unfortunately, this set of reactions did not work well because most of the

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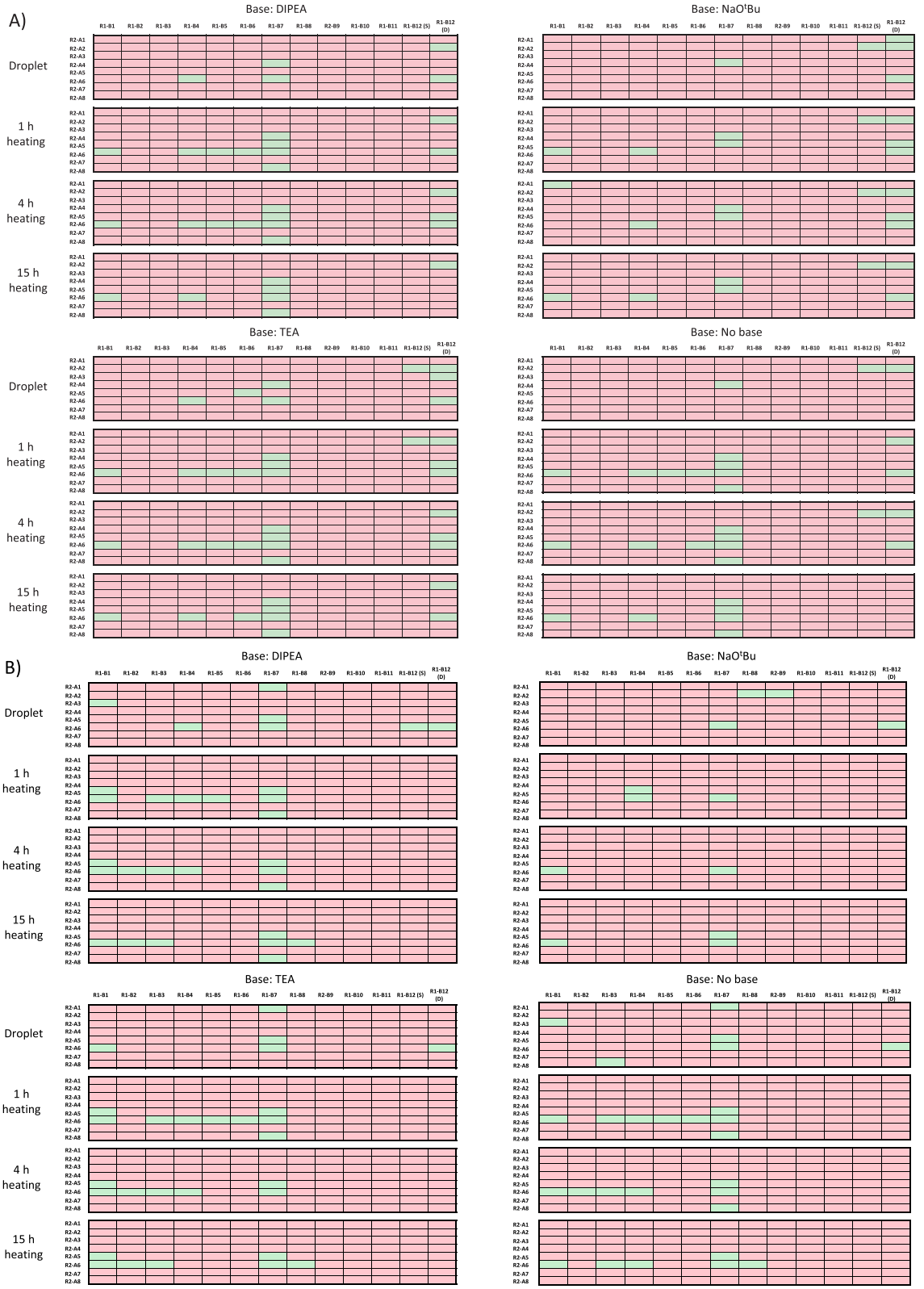

Figure 2. Heat map of 1536 reactions (768 in droplet/thin film and 768 in bulk microtiter at three time points) from round 2 of the S_NAr HTE using MeOH with 1% FA as the DESI spray solvent and NMP as the reaction solvent. (A) Droplet/thin film and bulk microtiter at 150 °C. (B) Droplet/thin film and bulk microtiter at 200 °C. Green cells represent successful reactions (average product intensity ≥ 150 counts). Red cells represent unsuccessful reactions (average product intensity < 150 counts).

amines were more electron-deficient than the round 1 amines, thus making them less nucleophilic toward the addition step. Only thiophen-2-ylmethanamine (**R2-A5**) and 2-morpholinoethan-1-amine (**R2-A6**) were comparatively better than the other amines because of their electron donating moiety. Again, **R1-B4**, **R1-B5**, **R1-B6**, **R1-B7**, and **R1-B12** worked better due

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Table 3. Summary of Product Outcomes (96 reactions) Round 2[a]

	DIPEA				NaO^{t}Bu				TEA				no base			
	DESI	bulk			DESI	bulk			DESI	bulk			DESI	bulk		
time (h)		1	4	15		1	4	15		1	4	15		1	4	15
150 °C	4	8	9	6	4	8	7	7	7	11	11	9	3	11	9	6
200 °C	7	8	9	6	4	3	2	3	5	9	7	7	6	8	7	6

[a]The bulk reaction results are reported for three different time points. Here, droplet experiments were replicates that were done in two different days.

to the presence of their strong electron-withdrawing nitro and chloro groups.

Table 3 summarizes round 2 of the S_NAr HTE reactions for both the DESI and bulk microtiter formats at different time points. Only 18 reactions worked in DESI at 150 °C, whereas 38 reactions worked in bulk after 1 h heating. Efforts to push the reaction at higher temperatures and longer times (200 °C for 15 h), did not improve the outcome (Figure 2B). Heating helped to promote reactions with the most reactive aryl halides (**R1-B1** through **R1B7**), with the most reactive amine being 2-morpholinoethan-1-amine, **R2-A6**; however, very high heating appeared to promote product degradation. Since most of the amines were much less nucleophilic, higher reaction temperatures did not help the reaction even after prolonged reaction times at higher temperature (see Tables SI1−4 for detailed peak intensity values).

Correlation Between DESI and Bulk Reactions. Figure 3 depicts the product intensities in the heated bulk microtiter

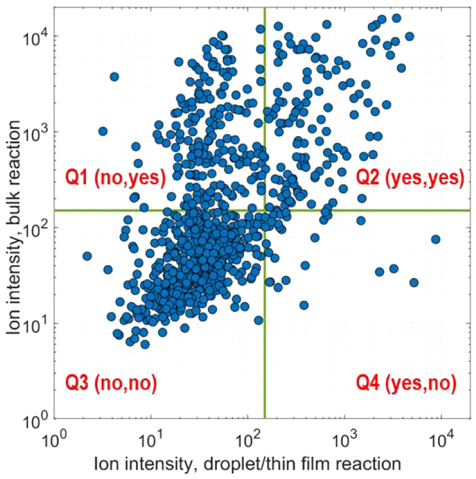

Figure 3. Correlation plot for the comparison of droplet/thin film and bulk data from 831 unique S_NAr products in round 1. Q1: 186 points. Q2: 124 points. Q3: 491 points. Q4: 30 points.

reactions of round 1 as a function of product intensities obtained in DESI. This correlation plot gives a visual representation of the agreement between the droplet/thin film and bulk reactions. Because a comprehensive statistical correlation analysis is beyond the scope of this study, this figure shows that a simple threshold intensity analysis provides a binary "yes"−"no" information space and splits the correlation plot into four important quadrants. Q2 and Q3 are the regions of good agreement between droplet/thin film and bulk microtiter outcomes. The reactions in Q1 are positives in bulk microtiter and negative in droplet/thin film. In the context of droplet reaction-guided bulk reaction design, these reactions would have been missed. Q4 points are positives in droplet/thin film but negatives in bulk microtiter. This later case is also misguiding, since these positive droplet reactions outcomes do not translate as positives under bulk reaction conditions. Possible reasons for these differences are summarized in Table 4.

Table 4. Possible Reasons for Discrepancy between Droplet/Thin Film and Bulk Microtiter Reaction Observations

mechanism	from	to	result
thermal degradation in bulk	Q2	Q4	false positive
incomplete bulk reaction (by reaction time or temperature)	Q2	Q4	false positive
thermodynamic control in bulk	Q3	Q1/Q2	false negative

Round 2, containing bulk microtiter reaction data at two temperatures and three reaction times, enabled a more detailed comparison of droplet/thin film and bulk microtiter reactions. It also reveals why there are conflicting findings in reaction outcomes (i.e., Q1 and Q4). For a bulk microtiter reaction, the reaction time and temperature are among the most important variables; however, the droplet/thin film reaction format cannot reliably approximate these conditions. For example, reaction A (Figure 4) showed only positive hits at 150 °C, but only negatives at 200 °C, whereas the opposite behavior is

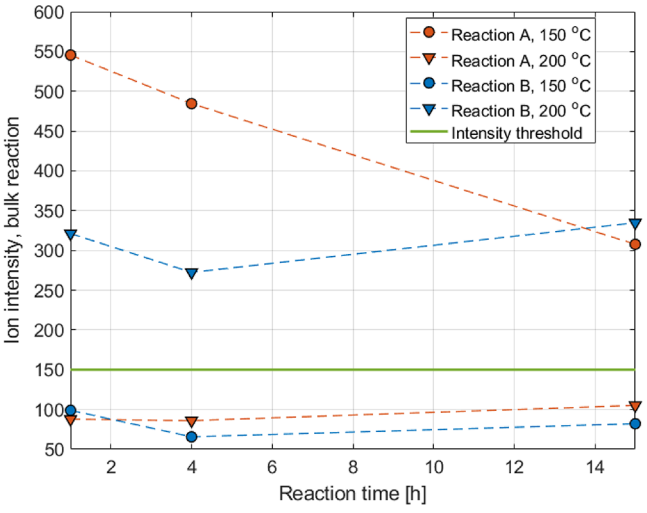

Figure 4. Two representative examples of heated bulk reaction outcomes showing the negative impact of thermal degradation (reaction A) and the positive effects of heating (reaction B). Reaction A: R2-A6, R1-B12(D); base, NaOtBu in NMP. Reaction B: R2-A6, R1-B3; base, DIPEA in NMP. The dashed lines are only to guide the eye.

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observed in the case of reaction B. Hence, in 50% of these bulk reactions, there is a discrepancy between the observed outcomes for droplet/thin film DESI and bulk microtiter reactions. Ideally, the optimum droplet/thin film and optimum bulk conditions should be comparable.

Reaction A is an example of thermal degradation. The reactions at 200 °C were all negatives, and although there are only positives at 150 °C, the product intensity decreases with time. Since this reaction was a "yes" in the droplet/thin film format, the thermal decomposition explains some of the false positives in that the product is detectable in DESI but is potentially degraded before the bulk microtiter experiment is terminated and assayed.

In sharp contrast, reaction B in bulk showed positives at high temperature (200 °C) and negatives at lower temperature (150 °C), and this reaction was a "no" under droplet/thin film conditions. The second S_NAr data set contains 13 such reactions (bulk reactions with more "yes" outcomes at 200 °C than at 150 °C, which were all negatives under droplet/thin film conditions). One of the proposed mechanisms of reaction acceleration is the lowering of the activation energy barrier, which is a kinetic effect.[13] Hence, an explanation for some false negatives can be that the high temperature in bulk might have shifted the chemical equilibrium toward product.

Microfluidic Evaluation. After identifying reaction hotspots from HTE, we tested some of the positive DESI findings via flow reaction conditions (Figure 5, Table 5) to check the validity of the high-throughput results. In these microfluidic experiments, the reactions were run at 30 s, 1, 3, and 5 min residence times at 100 and 150 °C using a 1:1 ratio of amines and aryl halides in NMP (Figure 3). DIPEA (2.5 equiv) was used as base since it showed the most promising results for both rounds 1 and 2. Reactions in 1,4-dioxane were not possible in flow because of the low solubility of the base in this solvent, resulting in reactor clogging.

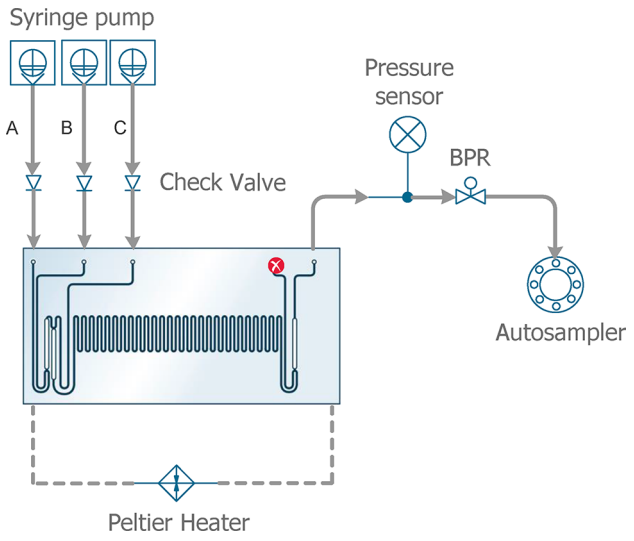

Figure 5. Continuous S_NAr reactions in flow using a Chemtrix glass chip reactor, SOR 3225: A = amine; B = aryl halide; C = DIPEA.

Table 5. Summary of S_NAr Reactions in Flow[a]

amines (μL/min)	aryl halides (μL/min)	base (μL/min)	residence time T_r (min)	temperature (°C)
6.67	6.67	6.67	0.5	100/150
3.33	3.33	3.33	1	100/150
1.11	1.11	1.11	3	100/150
0.67	0.67	0.67	5	100/150

[a]Chemtrix Reactor Chip: 3225. Reactor volume: 10 μL. Pressure: Ambient pressure.

Formation of the expected products in flow was confirmed by TLC and electrospray ionization-mass spectrometry (ESI-MS). We also found that the results of the microfluidic reactions were comparable to bulk and droplet screening experiments. Scheme 4 shows the "yes" reactions from HTE that were conducted in flow, including amines with EWGs that were found to produce successful reactions. The reaction of 4-chloro-6-ethyl-5-fluoropyrimidine (**R1-B7**) with 3-(2-methylpiperidin-1-yl)propan-1-amine (**R1-A7**) and 1-methylpiperazine (**R1-A4**) always produced the S_NAr product. Since 1-methyl-1*H*-imidazole (**R1-A2**) does not have a reactive amine site, it is not surprising that it did not participate in an S_NAr reaction. It should be noted that there is a possibility of obtaining a false positive result in MS for the reaction between 1-methyl-1*H*-imidazole (**R1-A2**) and 4-bromo-*N*,*N*-diethylaniline (**R1-B11**) because the *m*/*z* of the aryl halide starting material M+2 peak and the product *m*/*z* are same. Despite this, we were able to confirm that no product was formed from this reaction because the ratio of the bromine isotope peaks intensities always remained in a 1:1 ratio. 2-Morpholinoethan-1-amine, **R2-A6**, was the only reactive amine in the round 2 reaction set; continuous flow conditions also showed that this amine produced a positive result. We also examined two negative outcomes identified by HTE and found almost no product peak when those reaction conditions were evaluated under continuous flow (Scheme 5, see SI for spectra).

CONCLUSION

This investigation used a robotic technique to execute S_NAr reactions in 96-well arrays that were coupled with fast DESI-MS analysis to boost the speed of reaction optimization for the preparation of biologically important synthons. Extremely high throughputs can potentially be achieved using DESI because both synthesis and analysis can occur simultaneously; however, in the case of the S_NAr transformations studied herein, we found that many reactions required incubation at elevated temperatures to observe product. Even with the added time of incubation, the analysis times reachable with DESI (~3.5 s/sample) still results in sample throughputs that far exceed traditional techniques. A total of 16 amines and 13 aryl halides were used for HTE evaluation, yielding 1536 unique reactions each in droplet mode and bulk microtiter modes using four different bases in two different solvents. The outcome of 3072 individual reactions in this HTE campaign produced a total of 170 successful droplet reactions and 351 successful bulk microtiter reactions in a total experimental time of approximately 3 h. The expected impact of electron donating and withdrawing substituents on S_NAr reaction outcomes were observed in our HTE. A few of the positive and negative reactions identified by HTE were evaluated under continuous flow conditions. Those findings revealed that the positive conditions identified by HTE were true positives and the same was true for negative reaction conditions. Although many unsuccessful reaction conditions were identified by HTE, these negative results are highly valuable in that they can support machine learning efforts.[44,45] Since negative data is rarely published, the resulting gaps in the data available impedes the progress of groups trying to develop machine learning

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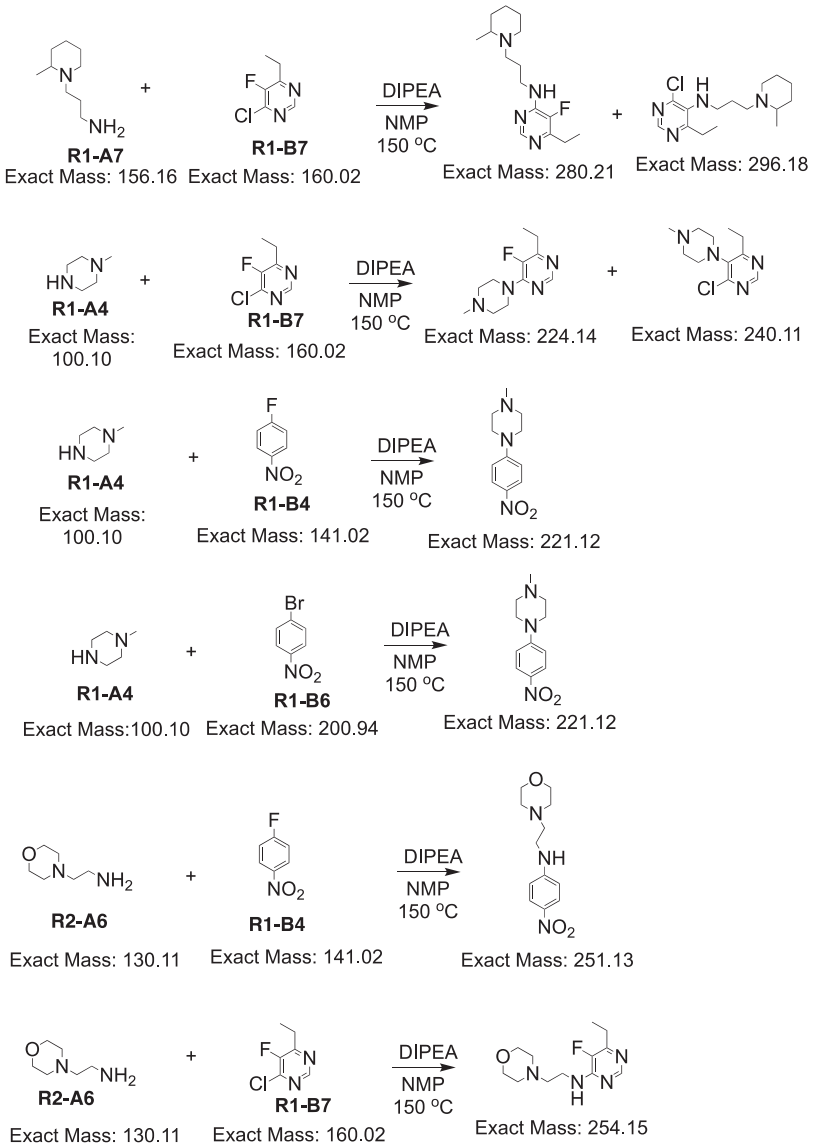
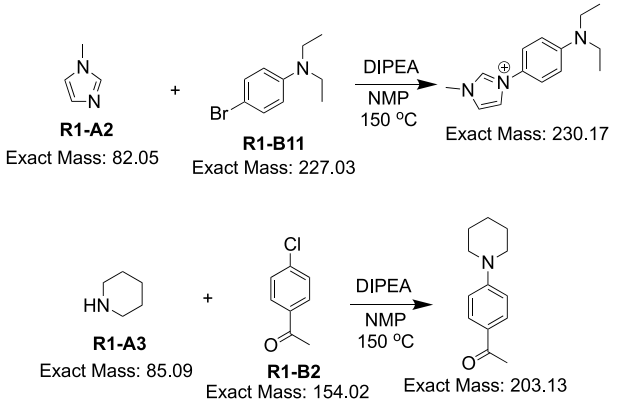
Scheme 4. Microfluidic Evaluation of Select "Yes" Reactions from the HTE Study



Scheme 5. Microfluidic Evaluation of Two "No" Reactions from the HTE



algorithms that can predict the success or failure of organic reactions.

The power of HTE in both the droplet/thin film and bulk microtiter reaction modes enables chemists to rapidly perform large arrays of rationally designed experiments and rapidly identify the most important reaction parameters for faster optimization of microfluidic reactions while also eliminating wasted effort spent exploring failed reaction conditions. Moreover, it makes it possible to derive multidimensional hypotheses that can be explained from easily collected huge data sets. Increasing the number of successful reactions can facilitate the population of libraries with more compounds for physicochemical and biological evaluation. Further, by applying this process to other common important class of reactions, it may accelerate library synthesis and the identification of optimal conditions for challenging substrates, suggesting that this approach is an important new tool in the repertoire of the synthetic chemist.

EXPERIMENTAL SECTION

All chemicals and reagents were purchased from Sigma-Aldrich (St Louis, Missouri) and used without any purification.

High-Throughput Reaction Conditions. High-throughput S_NAr experimentation in bulk was performed in 96-well metal block assemblies (Analytical Sales and Services, Inc., NJ,

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USA). The reaction mixtures were prepared in 1 mL glass inserts of the 96-well metal block. All the reagent transfers and mixing were performed using a Beckman Coulter i7 liquid handling robot. The stock solutions were 111 mM for amines and aryl halides, and the base stock solution concentration was 1.25 M in NMP or 1,4-dioxane. The final reaction concentrations were 50 mM (1 equiv) for both the amines and aryl halides and 125 mM (2.5 equiv) for the bases. All solutions were prepared in appropriate solvent and added to the 96-well plate in a ratio of 9: 9: 2 (amine: aryl halide: base). Additional solvent was used instead of base for the "no base" condition. For DESI-MS HTE, 384-well plates were prepared from the 96-well plates using the robot; a 384 pin tool was used to transfer the final reagent mixtures (50 nL) onto PTFE slides.

For bulk microtiter HTE, the plates were heated in a customized heating block at 150 or 200 °C for varying times. The cover on top of the glass inserts (top of the metal block) was made using a chemically resistant perfluoroalkoxy (PFA) film. Double silicone rubber mats were used on top of the PFA film, providing a tight seal that enables solution heating above the boiling point with less than 5% solvent loss and no cross talk between wells. After heating, the plates were cooled to room temperature, and loaded back onto the deck of the liquid handling robot to prepare 384-well plates. The reaction mixtures were pinned onto the same DESI slide as described above before and after heating using the same transfer method.

Liquid Handling Robot. Samples in 96-well aluminum blocks fitted with glass vial liners (Analytical Sales and Services, Inc., NJ, USA) or 384-well polypropylene plates (Analytical Sales and Services, Inc., NJ, USA) were prepared both for DESI and bulk HTE using a Biomek i7 (Beckman Coulter, Inc., Indianapolis, IN) liquid handling robot. A 384-tip head was used to transfer a single volume of 384 samples under the same speed of aspiration and dispensing conditions. Also, the heights of pipetting at the source and destination positions, pattern of pipetting, etc. remained constant for each transfer. An 8-channel head provided more flexibility in the amount of liquid transferred. Moreover, the 8-channel tip head provided better flexibility in terms of the layout of source and destination platforms, speed, pipetting height, and reaction stoichiometry. The i7 deck is also capable of accommodating all necessary labware including robotic tips, plates, reservoirs, etc., for assembling one reaction step. Chemically resistant polypropylene and disposables tips (Beckman Coulter, Inc., Indianapolis, IN) were used to make the reaction mixtures. The reservoirs of reagents solutions were the polypropylene multiwell plates and reservoirs, as well as custom-made Teflon reservoirs. Development of new transfer methods and validation were done using the Biomek point-and-click programming tool.

Customized Heating Block. Home built heating devices made of aluminum heater blocks containing four, 100 W cartridge heaters were fabricated to accommodate standard size 96-well plates. A CNi series temperature controller (Omega Engineering) enabled precise temperature control and a solid-state relay was used to modulate the 120 Vac power to the heaters.[15] The heating blocks tolerate temperatures ranging from −20 to 200 °C.

DESI-MS Analysis. DESI-MS analysis was performed following the previously published method of Wleklinski et al.[9] However, in this work, the density of reaction spots was 3072 spots/plate instead of 6144/plate. The Biomek i7 robot was used to prepare the DESI slide using reagents that were pipetted into standard polypropylene 384-well plates. Porous polytetrafluoroethylene (PTFE) sheets (EMD, Millipore Fluoropore, Saint-Gobain) were glued (Scotch Spray mount) onto glass slides (Foxx Life Sciences) to make the DESI slides. No signs of interference from the glue were observed. The reagents were mixed, and rhodamine B dye in a separate reservoir was added to the robotic deck as a fiducial marker. The liquids (50 nL) were deposited onto a porous PTFE surface using the magnetic pin tool at 3072 spot densities. A linear ion trap mass spectrometer (LTQ XL; Thermo Scientific, San Jose, CA) equipped with a commercial DESI-imaging source (DESI 2D source, Prosolia Inc., Indianapolis, IN) was used to collect the DESI-MS data. Xcalibur v.3.0 software was used to control the instrument and run the worklists for DESI-MS data acquisition. The DESI spray angle was 56° using MeOH or MeOH with 1% formic acid (FA) as spray solvent and with an applied voltage of 5 kV. Mass spectra were collected in positive ion mode over the m/z range of 50−500. The DESI-MS imaging lateral resolution was 350 μm. This was achieved using a stage speed of 4376 μm/s and an instrument scan time of 80 ms. The time to acquire data from one DESI slide was approximately 3 h, resulting in an analysis time of ∼3.5 s per reaction mixture. For data processing, data were visualized using in-house software designed[9] to automatically search for the m/z values of reactants, intermediates, and byproducts. The analysis using the in-house software generates a heat map indicating "yes/no" output for each spot on the PTFE surface of the DESI slide.

DESI-MS Analysis Software (CHRIS). The Chemical Reaction Integrated Screening (CHRIS) tool is an in-house software suite developed to automatically control the DESI system (mass spectrometer, solvents system, and Prosolia DESI 2D stage) and search the captured data for m/z values that correspond to the starting materials, intermediates, byproducts, and products. CHRIS generates a yes/no report, through a web interface and displays the mass spectrum of any spot, as well as spreadsheets with the intensity for the selected molecules, the possible contaminants, or unknown byproducts as guided by the user.

Microfluidic System. All microfluidic validation reactions were performed using a Labtrix S1 system (Chemtrix, Ltd., Netherlands). The system was described previously in Jaman et al.[15] The micro reactor 3225 is made of glass and used for all conducted reactions. The staggered orientated micro reactor (SOR) chip 3225 (four inlets and one outlet, volume 10 μL) have channel width 300 μm and channel depth 120 μm. The Labtrix unit is enabled to pump five syringes into the microreactor positioned on a heating and cooling unit. All the gastight glass syringes were bought separately from Hamilton Company (Hamilton, Reno, Nevada). All operations are controlled via a ChemTrix GUI software, connected to the Labtrix S1 casing using a USB cable.

Microfluidic Reaction Conditions. Solutions of amines (100 mM, 1 equiv) and aryl halides (100 mM, 1 equiv) in NMP were loaded individually into two separate 1 mL Hamilton gastight glass syringes (Hamilton Company, Reno, NV). DIPEA (150 mM, 1.5 equiv) solution in NMP was loaded into another 1 mL Hamilton gastight glass syringe. Each solution was continuously dispensed into the SOR 3225 reactor to engage the reactants. All the S_NAr reactions were run at 100 and 150 °C using residence times of 30 s, 1 min, 3 min, and 5 min. The products were collected without quenching

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and stored at −80 °C. TLC analyses were performed at the end of the reactions and the findings confirmed by subsequent ESI-MS analysis after extraction in ether and dilution into methanol.

Analysis of Microfluidic Reactions. A Thermo Fisher TSQ Quantum Access MAX mass spectrometer connected to a Dionex Ultimate 3000 Series Pump and WPS-3000 Autosampler (Thermo Fisher Scientific, Waltham, MA) was used to acquire electrospray ionization mass spectra (ESI-MS) of the samples. The analysis was performed in full scan mode, monitoring each analysis in both positive and negative ion modes. The optimized parameters for the ESI source and MS are as follows: spraying solvent, MeOH; spray voltage +5 kV (positive mode) and −5.0 kV (negative mode); capillary temperature, 250 °C; sheath gas pressure, 20; scan time, 0.5 s; Q1 peak width (fwhm), 0.70 Th; micro scans, 1. The autosampler settings were as follows: MS acquire time, 2 min; sample injection volume, 1 μL. The data from MS spectrometer was processed using Thermo Fisher Xcalibur software.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acscombsci.9b00212.

Methodological descriptions, DESI-MS peak intensity data from droplet and bulk microtiter HTE experiments, and time-/temperature-dependent mass spectral data from S_NAr reactions in flow appear (PDF)

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Author Contributions

Z.J., D.L.L., R.G.C., and D.H.T. designed the experiment. Z.J., D.A., and L.A. prepared the reaction mixtures. Z.J., D.L.L., B.S., and T.J.P.S. analyzed the data. Z.J., D.L.L., R.G.C., and D.H.T. wrote the manuscript. R.G.C. and D.H.T. secured the project funding.

Notes

The authors declare no competing financial interest.

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