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Multistep Flow Synthesis of Diazepam Guided by Droplet-Accelerated Reaction Screening with Mechanistic Insights from Rapid Mass Spectrometry Analysis

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Supporting Information

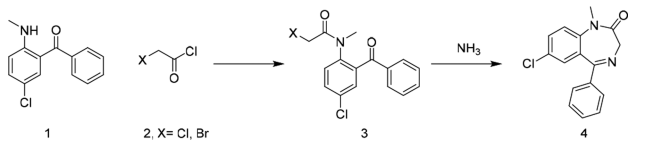
ABSTRACT: Electrospray and Leidenfrost droplet accelerated reactions were used as a predictive tool for estimating the outcome of microfluidic synthesis as demonstrated by Wleklinski et al. Rapid analysis by electrospray-mass spectrometry (ESI-MS) also provided immediate feedback on reaction outcomes in flow reactions. Significant reaction acceleration was observed in electrospray relative to the corresponding bulk reaction. This rapid reaction screening and analysis method has allowed for the detection of previously unreported outcomes in the reaction between 5-chloro-2-(methylamino)benzophenone and haloacetyl chloride (halo = Cl or Br) in the continuous synthesis of diazepam. In our current study, a more detailed extension of the previous work, we report acceleration factors that are solvent dependent; additional byproducts that were observed on the microfluidic scale that were absent in the droplet reactions. Gaining insight from this combined droplet and microfluidic screening/rapid ESI-MS analysis approach, we have helped guide the synthesis of diazepam and showcased the potential of this method as a reaction optimization and discovery tool. Informed by these new insights, diazepam was synthesized in a high-yield two-step continuous flow process.

1. INTRODUCTION

The potential for the efficient synthesis of active pharmaceutical ingredients (APIs) through continuous flow chemistry continues to draw interest from a broad range of disciplines throughout academia and industry.[1−5] Mass spectrometry (MS) has proven to be a useful tool in reaction monitoring as it allows monitoring of the kinetics and outcome of a reaction.[6] MS also helps in identifying reactive intermediates and hence in understanding the mechanistic details of a chemical reaction.[7] Additionally, MS can be used to study chemical reactions in droplets.[8] Previous studies have shown that most chemical reactions are accelerated in microdroplets formed by electrospray ionization (ESI) relative to the corresponding bulk reactions.[9,10] This acceleration is due, in part, to solvent evaporation and its effect on reagent concentration at the interface.[11] This rapid method of reaction screening can be useful in guiding microfluidic reactions and in scale up. An alternative way of generating microdroplets for studying chemical reactions is by employing the Leidenfrost effect. This effect occurs when a solution is dropped onto a heated surface such as a glass Petri dish which is held at a temperature higher than the boiling point of the solvent used. As the droplet approaches the heated surface, the solvent begins to evaporate, and a layer of insulating vapor is formed around the droplet. This prevents rapid evaporation of the solvent and causes the droplet to levitate.[12] Although the droplets that are formed by this technique are larger than ESI droplets, they have some of the same properties; therefore, reactions in these droplets can also be a useful tool in guiding microfluidic transformations.

In the present study, we use the synthesis of diazepam (Scheme 1) as a model system to showcase how the droplet screening demonstrated by Wleklinski et al.[13] guided the continuous synthesis of diazepam. Since a key step in this pathway involves N-acylation, these observations may have a more general bearing on N-acylation reactions in the synthesis of common bioactive molecules.[14] Although microdroplet conditions do not always directly translate into microfluidic scale conditions, this method can serve as a rapid yes/no prediction tool for the likelihood of a productive microfluidic reaction. Flow chemistry systems, coupled with online monitoring by ESI-MS, enable rapid screening of reaction conditions with real-time feedback. Not only does this allow for facile and efficient synthesis of APIs, but it also brings an opportunity for new insights into reaction pathways and byproduct formation. Diazepam may be obtained in two synthetic steps, starting from the N-acylation reaction of 5-chloro-2-(methylamino)benzophenone **1** with 2-haloacetyl chloride **2** giving amide **3**. Subsequent treatment with ammonia

Scheme 1. Proposed Pathway for the Continuous Synthesis of Diazepam



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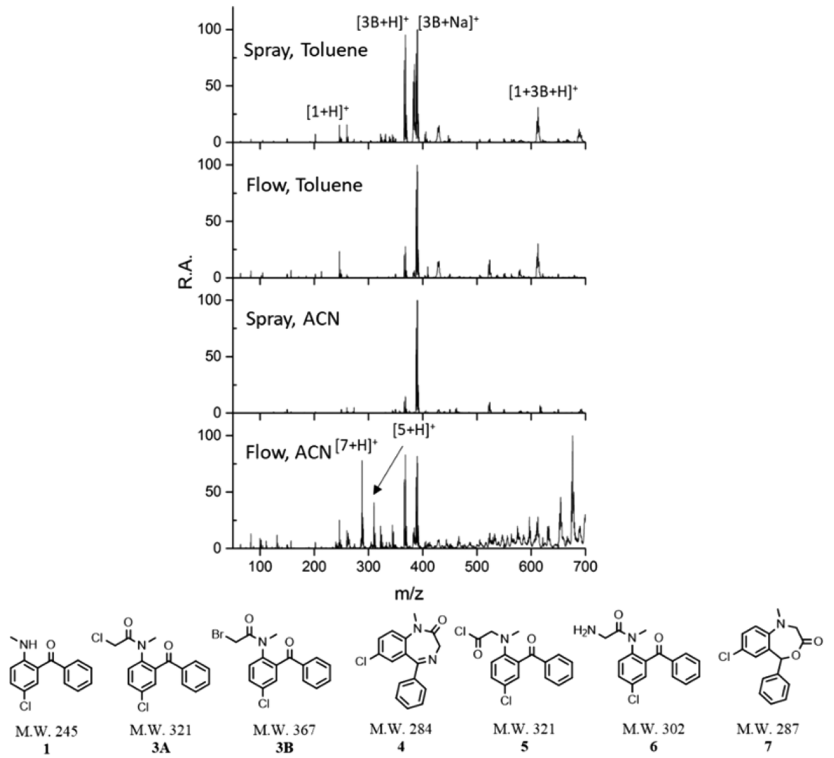

Figure 1. N-Acylation screen. Synthesis of **3** in using bromoacetyl chloride—comparison of spray and flow in toluene and acetonitrile.

then results in cyclization, giving diazepam **4** (Scheme 1). For each reaction screened in droplets or examined in flow reaction systems, the outcome was immediately ascertained by ESI-MS analysis.

2. RESULTS AND DISCUSSION

2.1. N-Acylation Reaction Screen. Reaction screening began with examination of the N-acylation step in ESI droplets, Leidenfrost droplets, bulk, and flow reaction systems. Initial ESI spray (offline) reaction screening across several solvents [dimethylacetamide (DMA), tetrahydrofuran (THF), dimethylformamide (DMF), acetonitrile (ACN), *N*-methylpyrrolidone (NMP), and toluene] revealed significant acceleration in ACN (~35×) and toluene (~100×) relative to a 30 min screen of the reaction in bulk at the same initial concentrations (Figure 1, Figure S1, Table 1). Although the spray experiment was performed in different solvents, the final extraction before analysis was done in ACN for all experiments.

Table 1. Accelerations Factors for the N-Acylation Reaction in the Synthesis of Diazepam

		acceleration factor[a]	
solvent	starting material	ESI spray	Leidenfrost
acetonitrile	chloroacetyl chloride	35	25
	bromoacetyl chloride	29	24
toluene	chloroacetyl chloride	97	14
	bromoacetyl chloride	38	34

[a]Acceleration factors reported in Table 1 are apparent acceleration factors given by the ratio of the mass spectrometry signals for the products relative to the starting materials (i.e., the conversion ratio) in the micro droplets relative to the bulk. This is not the same as the ratio of the rate constants for the two media.

The screening of flow reaction conditions for the first step also began with a solvent screen. NMP and DMF performed poorly, giving limited conversion and significant impurities. N-Acylation occurred rapidly and with good conversion in toluene, whereas in ACN, the formation of side products (**5**, **7**) arising from the S_N2 reaction pathway was observed (Figure 1).[13] In addition to solvents, temperatures and residence times were varied (Table S3). With increasing temperature, in most cases, conversion was poorer due to increasingly prevalent side reactions. By exchanging chloroacetyl chloride for bromoacetyl chloride, we obtained evidence of an alternative reaction pathway (previously reported by Wleklinski et al.).[13] The molecular weight of the expected product of this N-acylation reaction using bromoacetyl bromide is 367; however, we saw an additional peak at m/z 322. This corresponds to a loss of bromine rather than chlorine, as expected from N-acylation. A mixture of the S_N2 and N-acylation products (from the flow experiment) was isolated by column chromatography, and though they could not readily be separated from one another, NMR analysis showed two distinct sets of peaks at 3.7 and 3.9 ppm, corresponding to the methylene protons of the N-acylation and S_N2 products, respectively (Figure 2, Figure S2). Guided by these observations regarding the mechanism of the reaction where one solvent favored the more desired pathway and products, we determined that toluene was the optimal solvent for the continuous synthesis of diazepam.

In addition to this S_N2 product, we also observed a peak at m/z 288 in our flow experiments, consistent with ring closure to produce a seven-membered lactone. We propose that this m/z 288 compound (mol. wt. 287) is a result of an initial S_N2 reaction, followed by nucleophilic attack by the carbonyl on the acyl chloride to form the seven-membered ring. ^{1}H and ^{13}CNMR analysis confirmed this proposed structure (Figures S3 and S4, respectively). This result suggests that the selection

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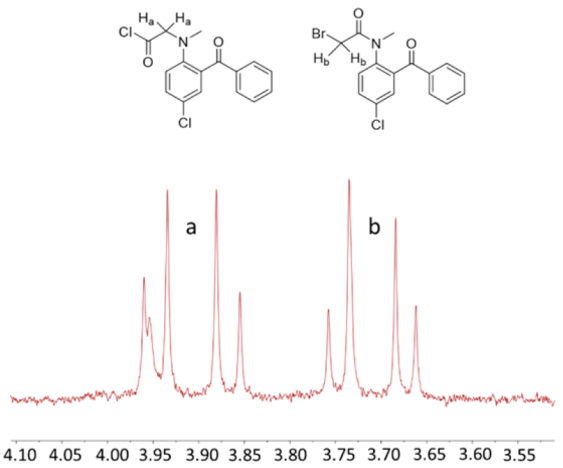

Figure 2. Methylene proton ^{1}H NMR signals of the S_N2 and N-acylation product mixture.

of reaction solvent and halide can be used to exert control over the reaction outcome in a microfluidic system. It is noteworthy that these alternative outcomes were observed in the microfluidic experiment but were absent in the corresponding droplet experiments. We believe that this is due to the high temperature and pressure conditions accessible in microfluidic reactions, but absent in droplet reactions, making alternative reaction pathways with higher energy requirements more likely.

2.2. Cyclization Reaction Screen. Previous screening of the cyclization reaction space revealed that a greater concentration of ammonia was required to form diazepam in Leidenfrost droplets (Figure 3). Reaction acceleration was observed in these droplet reactions, and they corroborated the microfluidic screen. A microfluidic screen of the second step from the N-acylation product **3A**, prepared previously, was carried out prior to attempting both steps in continuous flow. The solvent screen was limited by the low solubility of the chloro version of the intermediate (previously prepared in batch). For each solvent, varying temperatures and residence times were screened. The material was insoluble at a target concentration of 250 μM in ACN and toluene but dissolved well in NMP at this concentration. Fortunately, a good conversion to diazepam was observed in NMP (Table S3). Another interesting observation when studying this step was the appearance of a peak at m/z 303. We anticipated that this might represent a substitution of nitrogen at the methyl position. Due to the appearance of this peak in the MS at lower temperatures and residence times, and disappearance at higher temperatures, we also believed that it might be an intermediate in the diazepam synthesis. LC-MS analysis revealed that, despite the previous MS observation, the quantity of this m/z 303 material remained relatively constant throughout the temperature and residence time screen, even as the quantity of diazepam steadily increased. This could be in part due to high ionization efficiency of the m/z 303 material drowning out the signal from other compounds present in the reaction mixture. This m/z 303 material (**6**) is believed to be a previously reported hydrolysis product of diazepam.[15]

2.3. Continuous Diazepam Synthesis. On the basis of these observations, we were able to develop a more complete understanding of the possible outcomes of each synthetic step of our synthetic route to diazepam. These possible reaction pathways are summarized in Scheme 2. Guided by this knowledge from our screening, we next attempted to optimize the synthesis of diazepam in two continuous steps. We used a two-chip reactor system to allow for finer control of

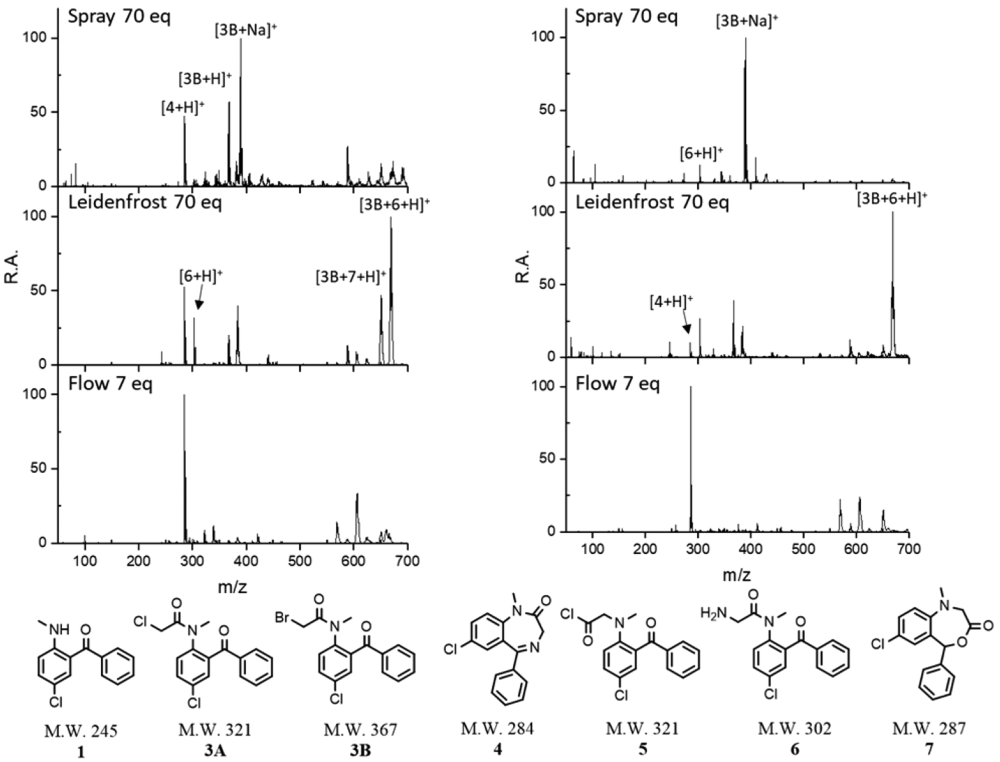

Figure 3. Cyclization reaction screen. Synthesis of diazepam, comparing ACN (left) and toluene (right) solvents in spray, Leidenfrost, and flow reactions using bromoacetyl chloride.

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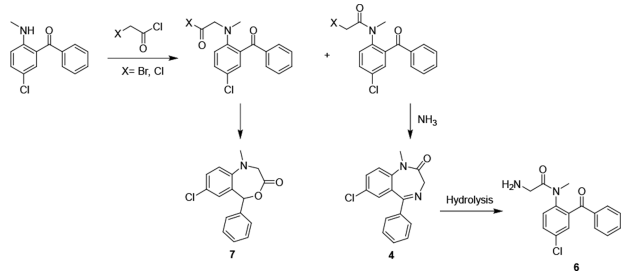
Scheme 2. Reaction Pathway to Diazepam and Its Byproducts Formed during Continuous Flow Reaction



temperature and residence time in each chip. The first chip combined 5-chloro-2-(methylamino)benzophenone and haloacetyl chloride in a 1:2 ratio, respectively (Figure 4, R1 and R2),

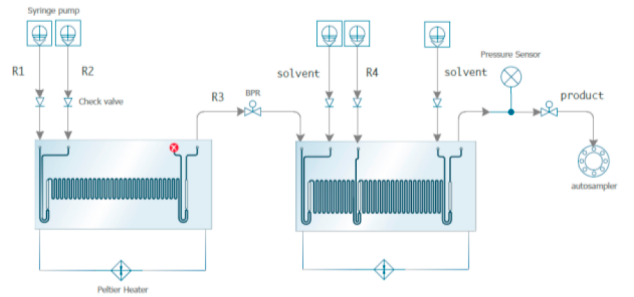


Figure 4. Reactor schematic for continuous diazepam synthesis.

before dilution of the resultant N-acylation product mixture (R3) with methanol and subsequent addition of ammonia/methanol (R4) in a 7-fold excess in the second chip.

Solvent screening was again limited by solubility, particularly upon addition of ammonia/methanol in the second step. To alleviate this problem, a dilution step after the first reaction step was incorporated to improve solubility. Good solubility was achieved using toluene for the first step and diluting 1:4 with methanol or NMP. The use of ACN in the first step and for dilution was also effective. As in the previous flow reaction screens, we varied temperature and residence time, as well as the selection of bromo- vs chloroacetyl chloride (Table S4). The results of this screen seemed to corroborate our previous observations, with bromoacetyl chloride resulting in some S_N2 product, particularly in ACN. Furthermore, the overall conversion from the intermediate to diazepam was higher when bromoacetyl chloride was used.

Yields for each reaction were determined using a quantitative ESI-MS/MS method. The optimum result was achieved using bromoacetyl chloride in the toluene/methanol solvent system, which yielded diazepam in 100% yield, based on our ESI-MS quantitation method. This was also the optimal solvent system in Leidenfrost and spray microdroplets (Table S1).

3. CONCLUSIONS

This study, using the diazepam synthesis as a model reaction, demonstrates the ability of MS analysis and droplet reactions to guide microfluidic synthesis. MS can be used not only as an analytical tool but can also serve as a quick way to predict reactivity and guide microscale synthesis. The use of spray and Leidenfrost droplet reactions as a screening step to guide the larger scale microfluidic screening proved a useful tool in predicting the overall outcome of a reaction. While some nuances observed in flow reactions were not observed in droplet experiments, these experiments still consistently provided a yes/no indication of the viability of a reaction. Further, we have demonstrated the continuous synthesis of diazepam in two steps in a microfluidic flow reactor. Our synthesis features the use of a mixed solvent system, as well as two microfluidic chips in sequence, allowing for optimized temperature control at each step. Additionally, we have identified previously unknown reaction pathways. These results showcase the possibility for microfluidic synthesis coupled with rapid ESI-MS analysis to identify previously unknown reaction pathways and optimize continuous synthesis of APIs.

4. EXPERIMENTAL SECTION

4.1. Reagents. Reagents were purchased from Sigma-Aldrich and used without further purification.

4.2. NMR Analysis. NMR samples were prepared by microscale SiO_2 column chromatography. Samples were analyzed using a Bruker AV-III-500-HD NMR spectrometer.

4.3. Mass Spectrometry. A mass spectral analysis of reaction products was performed using an LTQ ion trap mass spectrometer (Thermo Fisher Scientific, San Jose, CA) with nanoESI (nESI) ionization. All product samples (spray, Leidenfrost, or flow reactions) were diluted 1:100 into ACN before analysis, unless otherwise noted. The distance between the tip of the spray emitter and ion transfer capillary to the MS was kept constant at ca. 1 mm. Experiments were performed using borosilicate glass pulled to a ca. 1−3 μm aperture. A spray voltage of either positive or negative 2.0 kV was used for all analyses. Positive-ion mode was used for all chemical analyses, unless otherwise noted. Product ion (MS/MS) spectra were recorded using collision-induced dissociation (CID) with a normalized collision energy of 25 (manufacturer's unit).

4.4. Quantitative MS Analysis. An MS based calibration was made from mixtures of 0, 1×10^{-7}, 1×10^{-6}, 2.5×10^{-6}, and 8×10^{-6} M diazepam with 3.88×10^{-6} M diazepam-D3. Each point was measured with nESI in triplicate, and the calibration is based on the diazepam to diazepam-D3 ratio. Crude reaction samples were quantified by first diluting an appropriate amount (×10 000 typically) and then adding the same amount of internal standard. Each crude sample was diluted in duplicate and analyzed by nESI.

4.5. ESI Experiments. These experiments were performed by spraying the reaction mixture directly onto glass wool and then extracting the sprayed residue with ACN. The extract was diluted 1:100 then analyzed by MS. A home-built electrospray ionization source was used. Reagents 1 and 2 were premixed at concentrations of 100 mM and 200 mM, respectively, and loaded into a syringe. Offline spray was carried out at a flow rate of 10 μL/min, 100 psi N_2 sheath gas, and 5 kV voltage. The total spray time was 10 min. After MS analysis, the washed material was drawn back into the syringe and mixed with ammonia in methanol and then electrosprayed to synthesize diazepam.

4.6. Leidenfrost Droplet Experiments. This experiment was carried out on a hot plate with a heat setting of 540 °C (although the droplet temperature was much lower). Reagents 1 and 2 were premixed and loaded into a Pasteur pipet. The reaction mixture was then dropped onto a glass Petri dish that was placed on the hot plate. The reaction mixture was added in aliquots over a time period of about 2 min. After 2 min, the mixture was collected from the surface using a Pasteur pipet and then analyzed by MS after diluting 1:2.

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4.7. Microfluidic Experiments. All microfluidic reactions were carried out using a Chemtrix Labtrix S1 system, equipped with 3223 or 3224 reactor chips.

4.8. *N*-(2-Benzoyl-4-chlorophenyl)-2-halo-*N*-methylacetamide (3). Solutions (100 mM) of 5-halo-2-(methylamino)benzophenone (1 equiv) and of haloacetyl chloride (1 equiv) in toluene, ACN, DMF, or NMP were prepared. In the DMF reaction screen, 500 mM solutions were used; in the NMP reaction screens, 250 mM solutions of benzophenone and 500 mM (2 equiv) chloroacetyl chloride were used. A syringe was loaded with each of these two solutions and positioned on the first two inlets of a 10 μL Labtrix 3223 chip. A third syringe was loaded with toluene and positioned on the third port of the same chip as a diluent. The reaction was flowed with 30, 60, and 180 s residence times at temperatures of 50, 100, and 150 °C. Samples were collected and immediately analyzed by ESI-MS (1 μL of each sample was diluted with 99 μL of ACN, then loaded into a glass electrospray tip for analysis). Samples were saved and stored at −20 °C.

4.9. Diazepam (4) from *N*-(2-Benzoyl-4-chlorophenyl)-2-chloro-*N*-methylacetamide (3A). A 250 mM solution of *N*-(2-benzoyl-4-chlorophenyl)-2-chloro-*N*-methylacetamide (1 equiv) was prepared. Syringes were loaded with the prepared solution and with 7 N ammonia in methanol (7 equiv) and positioned on the first two inlets of a 10 μL Labtrix 3223 chip. A third syringe was loaded with NMP and positioned on the third port of the same chip as a diluent. The reaction was flowed with 30, 60, and 180 s residence times at temperatures of 50, 100, and 150 °C. Samples were collected and immediately analyzed by ESI-MS (1 μL of each sample was diluted with 99 μL of ACN, then loaded into a glass electrospray tip for analysis). Samples were saved and stored at −20 °C.

4.10. Diazepam (4) from 5-Halo-2-(methylamino)-benzophenone (1). Solutions (100 mM) of 5-halo-2-(methylamino)benzophenone (1 equiv) and 200 mM haloacetyl chloride (2 equiv) in toluene or ACN were prepared. Syringes were loaded with the prepared solutions and with 7 N ammonia in methanol (7 equiv). The syringes containing 5-halo-2-(methylamino)-benzophenone and haloacetyl chloride were positioned on the first two inlets of a 10 μL Labtrix 3223 chip. The mixture was flowed into a second 15 μL Labtrix 3224 chip through the first inlet. A syringe containing toluene, NMP, or ACN was positioned at the second inlet of the 3224 chip to dilute the reaction by 1:4. The syringe containing 7 N ammonia in methanol was positioned at the third inlet of the 3224 chip. A final syringe containing toluene, NMP, or ACN was positioned at the final inlet of the 3224 chip, flowing at a low flow rate of solvent to help avoid fouling at the outlet. The reaction was flowed with residence times of 1 + 0.32 s and 2 + 0.62 s (chip 1 + chip 2) and temperatures of 75 °C in chip 1 and 100, 110, 120, or 140 °C in the second chip. Samples were collected and immediately analyzed by ESI-MS (1 μL of each sample was diluted with 99 μL of ACN, then loaded into a glass electrospray tip for analysis). Samples were saved and stored at −20 °C.

4.11. Batch Synthesis of *N*-(2-Benzoyl-4-chlorophenyl)-2-chloro-*N*-methylacetamide (3A). Chloroacetyl chloride (0.39 mL, 4.9 mmol, 1 EQ) was added to a solution of 5-chloro-2-(methylamino)benzophenone (1.2 g, 4.9 mmol) in 100 mL NMP (50 mM). The reaction was heated to 90 °C for 40 min. The solution was then concentrated in vacuo, and the product was isolated via SiO_2 column chromatography in 68% yield. The structure was confirmed by ^{1}H NMR (Figure S5).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.oprd.7b00218.

Structure index, Figures S1−S5, Tables S1−S4 (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Adamo, A.; Beingessner, R. L.; Behnam, M.; Chen, J.; Jamison, T. F.; Jensen, K. F.; Monbaliu, J. M.; Myerson, A. S.; Revalor, E. M.; Snead, D. R.; Stelzer, T.; Weeranoppanant, N.; Wong, S. Y.; Zhang, P. *Science* **2016**, *352* (6281), 61−67.

(2) Bédard, A.; Longstreet, A. R.; Britton, J.; Wang, Y.; Moriguchi, H.; Hicklin, R. W.; Green, W. H.; Jamison, T. F. *Bioor. Med. Chem.* **2017**, 10.1016/j.bmc.2017.02.002.

(3) Baumann, M.; Baxendale, I. R. *Beilstein J. Org. Chem.* **2015**, *11*, 1194−1219.

(4) Malet-Sanz, L.; Susanne, F. *J. Med. Chem.* **2012**, *55*, 4062−4098.

(5) McQuade, D. T.; Seeberger, P. H. *J. Org. Chem.* **2013**, *78*, 6384−6389.

(6) Chen, P. *Angew. Chem., Int. Ed.* **2003**, *42*, 2832−2847.

(7) Santos, L. S.; Rosso, G. B.; Pilli, R. A.; Eberlin, M. N. *J. Org. Chem.* **2007**, *72*, 5809−5812.

(8) Huang, G.; Li, G.; Ducan, J.; Ouyang, Z.; Cooks, R. G. *Angew. Chem., Int. Ed.* **2011**, *50*, 2503−2506.

(9) Banerjee, S.; Zare, R. N. *Angew. Chem., Int. Ed.* **2015**, *54*, 14795−14799.

(10) Yan, X.; Bain, R. M.; Cooks, R. G. *Angew. Chem., Int. Ed.* **2016**, *55*, 12960.

(11) Bain, R. M.; Pulliam, C. J.; Cooks, R. G. *Chem. Sci.* **2015**, *6*, 397−401.

(12) Bain, R. M.; Pulliam, C. J.; Thery, F.; Cooks, R. G. *Angew. Chem., Int. Ed.* **2016**, *55*, 10478−10482.

(13) Wleklinski, M.; Falcone, C. E.; Loren, B. P.; Jaman, Z.; Iyer, K.; Ewan, H. S.; Hyun, S. H.; Thompson, D. T.; Cooks, R. G. *Eur. J. Org. Chem.* **2016**, *33*, 5480−5484.

(14) Roughley, S. D.; Jordan, A. M. J. *J. Med. Chem.* **2011**, *54*, 3451−3479.

(15) Maslanka, A.; Krzek, J.; Szlosarczyk, M.; Zmudzki, P.; Wach, K. *Int. J. Pharm.* **2013**, *455*, 104−112.

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