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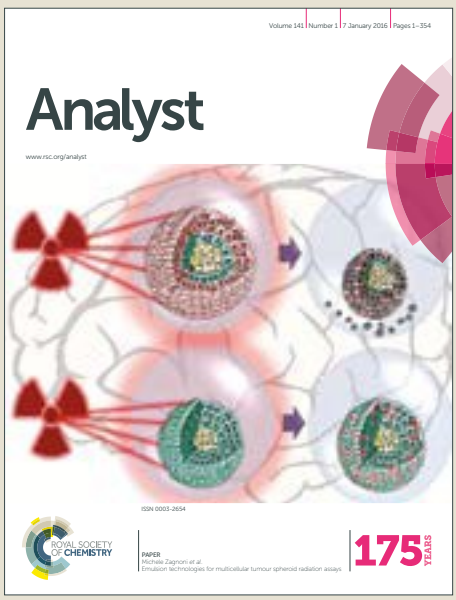

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Reaction Screening and Optimization of Continuous-Flow Atropine Synthesis by Preparative Electrospray Mass Spectrometry

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Abstract

Preparative electrospray (ES) exploits the acceleration of reactions in charged microdroplets to perform small scale chemical synthesis. In combination with on-line mass spectrometric (MS) analysis, it constitutes a rapid screening tool to select reagents to generate specific products. Successful reaction in preparative ES triggers a refined microfluidic reaction screening procedure which includes optimization for stoichiometry, temperature and residence time. We apply this combined approach to refining a flow synthesis of atropine. A successful preparative ES pathway for synthesis of the phenylacetyl ester intermediate, using tropine/HCl/phenylacetyl chloride, was optimized for solvent in both the preparative ES and in microfluidics flow systems and a base screen was conducted by both methods to increase atropine yield, increase percent conversion and to reduce byproducts. In preparative ES, the first step yielded 55% conversion (judged by MS) to intermediate and the second step yielded 47% conversion to atropine. When

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combined in two *discrete* steps in continuous-flow microfluidics, a 44% conversion of starting material and a 30% actual yield of atropine were achieved. When the reactions were *continuously* telescoped in a new form of preparative reactive extractive electrospray (EES), atropine was synthesized with 24% conversion. The corresponding continuous-flow microfluidics experiment gave 55% conversion with an average 34% yield in an 8 min residence time. This is the first in depth study utilizing telescoped preparative ES and the first use of dual ESI emitters for multistep synthesis.

Introduction

Continuous flow reactors are gaining traction in pharmaceutical and fine chemistry for the preparation of small and large molecules including active pharmaceutical ingredients (APIs).[1- 2] Key advantages of continuous flow are enhanced mass transfer, controlled flow, ease of integration, precise control of reaction temperature and reaction time, as well as high efficiency and safety.[3-4] Generally, reaction optimization and screening require significant investments in time and material.[5,6] Pharmaceutical production still utilizes a supply chain network where reagent shortages often occur due to use of multipurpose batch reactors which are operated in cycles and lengthy experiments may be required to identify optimize the intermediates in each of several reaction steps while minimizing carry-through of by-products.[7] Microreactors, used in conjunction with on-line nESI-MS analysis, speed up reaction optimization[8] and lower costs due to low material requirements and reduced waste generation.[9,10] The reduced channel widths, together with the exceptional mass-and heat-transfer capacity of these reactors, are responsible for decreasing reaction times. [11] With all of these advantages, it is expected that continuous flow processes should emerge as an important technique for API synthesis.[12-13] Indeed, many APIs have been synthesized continuously by incorporating small-scale work up techniques and formulation. Continuous flow synthesis of the APIs efaproxial, rimonabant[14], imatinib[15,16], ibuprofen[8], rufinamide[17], diphenhydramine hydrochloride[18], (E/Z)-Tamoxifen[19] have been reported., Two MS experiments are investigated here to further facilitate the development of continuous flow synthesis, (i) rapid screening using accelerated reactions in microdroplets or thin films and (ii) on-line continuous MS monitoring of the microfluidic reaction using inductive ESI.[20] The methodology differs from but can be compared to the use of drop-based microfluidics for screening analytes.[21]

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In preparative electrospray, accelerated chemical reactions occur in charged microdroplets, reducing reaction times.[22] Accelerated reactions in microdroplets have been studied using desorption electrospray ionization (DESI),[23-25] nanoDESI,[26] paper spray[27] and electrospray ionization (ESI)[28] as well as in levitated form.[29] These reactions have been employed to derivatize analytes for improved MS analysis,[22] in mechanistic studies, [30-32] to identify reaction intermediates[24, 33, 34] and to perform microscale synthesis.[35, 36] Accelerated droplet reactions can be monitored by on-line MS analysis and they can be used to prepare milligram quantities of material in minutes. [35] In preparative ES a reaction mixture is optimized for product formation by on-line MS analysis then electrosprayed off-line onto a collector surface. The material deposited is subsequently analyzed by extraction with solvent and off-line chemical analysis.

Extractive electrospray ionization (EESI) is an alternative spray ionization technique that utilizes two ESI emitters to allow collisions between droplets from two separate streams. One emitter nebulizes the sample and the other produces charged microdroplets of solvent.[37-44] Liquid-liquid extraction of analytes from the sample during microdroplet collisions leads to extraction of analyte into a solvent that is amenable to on-line mass spectrometric analysis. As used here, the second spray contains a reagent which can react with the intermediate products of the first sprayer to yield the desired final product. Note that a technique similar to EESI, droplet fusion, uses two separate ESI emitters to collide microdroplet reagents to form fused droplets containing both reagents. The reagents mix in the fused droplets and the reaction proceeds. Microdroplet fusion has been used to study the kinetics of phenolindophenol reduction by ascorbic acid, acid-induced cytochrome c unfolding, and HDX in bradykinin.[45] In a third multi ESI emitter technique, multichannel rotating electrospray ionization, ESI emitters nebulize volatile reagents that induce reactions in the gas phase and the resulting products are extracted by droplets from another ESI emitter.[46] Among these three methods, only EESI was used in this study.

Atropine is traditionally manufactured industrially by natural product extraction[47]. Atropine, the natural tropane alkaloid, exists as a racemic mixture of D-hyoscyamine and L-hyoscyamine and has both anticholinergic and antiparasympathetic properties.[48] The drug is included in the WHO list of essential medicines and the U.S Food and Drug Administration (FDA) has reported it to be among drugs which were in short supply during 2011-2014.[49] The

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total synthesis of atropine has previously been published using a batch process[50, 51, 52] and more recently using continuous flow.[49] This previous continuous flow process required multiple steps of purification by liquid-liquid extraction due to a variety of byproducts and this reduced the overall yield of atropine.[49,53] Reported here, is a rapid screening technique that utilizes preparative ES to screen pathways and microfluidics with on-line MS to optimize selected pathways.

Experimental

All chemicals and solvent were purchased from Sigma-Aldrich (St. Louis, Missouri) and used without any purification. pH = 10 buffer was purchased from Macron (Avantor Performance Materials,Center Valley, PA)

Mass Spectrometry

Samples were analyzed using a Thermo LTQ linear ion trap mass spectrometer (ThermoFisher Scientific, San Jose, CA, USA). NanoESI analysis in both positive and negative ion mode was performed using a 2.0 kV spray voltage. Other experimental parameters were: capillary temperature: 200C; tube lens (V): -85 V; capillary voltage: -20 V for positive ion mode and tube lens (V): 85 V; capillary voltage: 20V for negative ion mode. Tandem mass spectrometry was performed using an isolation window of 1.5 (*m/z* units) and 25% collision energy. The spectra were acquired with automatic gain control while averaging 3 micro-scans for each spectrum. Samples were prepared for nanoESI by diluting 100-fold in acetonitrile.

Microfluidics

All microfluidic syntheses were performed using a Labtrix S1 (Chemtrix, Ltd., Netherlands). A home built Peltier-controlled system coupled with the Chemtrix microfluidic platform allowed for multi-step reactions to be performed with control over the temperature in each chip.

HPLC-MS Analysis

Separations were performed on an Agilent 1100 HPLC system (Palo Alto, CA) using a Varian C18 Amide column (3 µm, 150 x 2.1 mm id) and 10 uL injection volume. Following the separation, the column effluent was introduced by positive mode electrospray ionization (ESI)

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into an Agilent MSD-TOF mass spectrometer. The percent conversion was calculated as the product signal divided by the sum of all the peaks in the spectra.

RP-UPLC Analysis

An isocratic reverse-phase ultra-high performance liquid chromatography method (RP-UPLC) using the PATROL UPLC Process Analysis System (Waters Corp.) was used to determine the yield of atropine in continuous flow. The detection of eluted atropine was accomplished using a dual-channel PDA detector at 190 and 225 nm in conjunction with ApexTrack analysis for integrating the atropine peak that was matched with an atropine standard chromatogram. Quantification was then performed via interpolation using a standard calibration curve at 225 nm. Three standard stock solutions of atropine sulfate were prepared.

NMR Analysis

1H-NMR and 13C-NMR samples were prepared by dissolving ~10 mg of sample in CDCl3 and spectra were acquired using a Bruker AV-III-500-HD NMR spectrometer (Billerica, Massachusetts, USA). The NMR data was analyzed using MestReNova 10.0 software.

Preparative Electrospray

A homebuilt electrospray source was enclosed in a polypropylene tube (15mL Falcon tube) with a small piece of glass wool for deposition. The reaction mixture was pumped through fused silica capillary at a rate of 10uL/min and a high voltage of 5kV is applied through the stainless steel needle of the 250uL Hamilton gastight syringe. A nitrogen sheath gas was used at 100psi. The sprayed droplets were collected on the glass wool and washed with a solvent for analysis by nanoESI or to be telescoped for the next step in the reaction.

Preparative Reactive EES

Two homebuilt electrospray sources were angled at 22.5^{o} with the intersection of the spray plumes occurring at a distance of 4 cm. The deposition surface of glass wool in a polypropylene tube was placed at varying distances from the intersection point. Each ES emitter was connected to two syringes via tubing and mixed in line with a mixing T. Each syringe had a flow rate of

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5uL/min with a total flow rate of 10uL/min for each ES emitter. The high voltage was applied through a platinum electrode in a T junction. A nitrogen sheath gas was used at 100psi. The sprayed droplets were collected on the gas wool and washed with a solvent for analysis by nanoESI.

Synthesis of Intermediates in Preparative ES

For the synthesis of intermediate **4**, 0.1mmoles of solid tropine **2** and 0.1mmoles of phenyl acetic acid or phenylacetyl chloride **5** were added to 100uL of aqueous 3M HCl or 100uL of 4M HCl in dioxane and sprayed for 5 minutes. For the synthesis of intermediate **4**, 0.1mmoles of solid tropine **2** and 0.1mmoles of phenyl acetic acid or phenylacetyl chloride **5** were added to 100uL of solvent and sprayed for 5 minutes. All deposited products were washed with 500uL ACN and analyzed via nanoESI-MS.

Synthesis of Intermediates in Microfluidics

A DMF solution of tropine **2** HCl in dioxane and phenylacetyl chloride **6** were individually loaded onto 1 mL ILS gas tight glass syringes. Each solution was in turn dispensed onto the SOR 3225 reactor to react and produce intermediate **4**. Similarly, a DMA solution of tropine **2** and phenylacetyl chloride **5** were loaded in a pair of 1 mL syringes and reacted onto the SOR 3227 reactor to prepare for intermediate **4**. The esterification reaction was run at 100°C, 150°C and 200°C in residence times of 1 min, 2 min, 5min, and 8 min. The intermediate was collected without quenching. The subsequent nESI-MS and HPLC-MS analyses were performed without further purification. By contrast, NMR analysis was performed after neutralization and extraction of the reaction mixture.

Synthesis of Atropine in Preparative ES

The first step product of the atropine synthesis was achieved by preparative ES spraying 200uL 1:1 0.5M tropine in DMA: 0.5M phenylacetyl chloride in DMA and then washing the deposited product from the glass wool with 500uL DMA. The second step of the atropine synthesis was performed by spraying 50uL of the following solution: 10uL Step 1 product, 30uL 36-37% formaldehyde solution in water and 30uL 1M base in DMA. The sample on the glass wool was

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quenched with 500uL water and the product was extracted with 100uL DCM. The preparative ESI product was analyzed via nanoESI.

Synthesis of Atropine by Preparative EES

The first ES emitter sprayed the step 1 reagents to form the intermediate and the first syringe contained 0.1M tropine in DMA and the second syringe contained 0.1M phenylacetyl chloride in DMA. The second ES emitter sprayed the second step reagents and the first syringe contained 1M 1,5-diazabicyclo[4.3.0] in DMA and the second syringe contained 36-37% formaldehyde solution in water. A total of 100uL of each reagent was sprayed for 20 minutes. The glass wool was quenched with 2mL water and the product was extracted with 100uL DCM. For analysis by nanoESI, 10uL of the DCM extract was diluted in 90uL of ACN.

Synthesis of Atropine by Microfluidics using Two Chips

DMA solution of intermediate **4** and aqueous formaldehyde solution with each of nine bases in turn, was loaded onto a pair of 1 mL glass syringes and delivered at temperatures starting from room temperature to 200°C. All the bases were diluted in DMA. The residence times of the reactions also varied from 10 sec to 8 minutes, depending on the base, in a Labtrix SOR reactor, such as the 3227, 3225, 3223 or 3222 reactor. Deionized water was loaded into the last 1 mL glass syringe and delivered to quench the reaction (see Supporting Information for details).

Continuous Microfluidic Synthesis of Atropine

The DMA solutions of tropine **2** (1.0 equiv) and phenylacetyl chloride **5** (1.0 equiv) were loaded into a pair of 1 mL glass syringes and delivered to SOR 3221 at 100°C in 2-min residence time. The solution from SOR 3221 was entered to the first inlet of SOR 3223. A DMA solution of 1,5-diazabicyclo[4.3.0] base and formaldehyde solutions (4.0 equiv) and water were loaded onto a pair of 1 mL glass syringes and delivered to SOR 3223 at 70°C in 6-min residence time.

Results and Discussion

Preparation of Intermediate Ester 4 by Electrospray

Mutiple reaction pathways for the synthesis of atropine (**1**) were explored using preparative ES with off-line product collection (Figure 1). In its simplest form, preparative ES gives a binary

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response as to whether a synthetic route does or does not yield product. A successful reaction in spray guides the choice of possible reactions to pursue in microfluidics. Four synthetic routes (scheme 1) were screened by preparative ES for formation of the atropine intermediate **4.** The first pathway (scheme 1a) explored the use of tropine **2**, phenyl acetic acid **3** and HCl in both water and in dioxane. The use of aqueous HCl yielded no observable intermediate **4** in the full scan MS, however the presence of product in low abundance was evident from the MS/MS spectrum recorded for ions of *m/*z 260, of the value corresponding to the protonated intermediate. The use of HCl in dioxane yielded a low but measurable signal for intermediate **4** in the full scan MS with a conversion of 1.4%. (conversion is defined as the abundance of the product ion divided by the sum of the abundances of the reagent, product, and byproduct ions). The second pathway (scheme 1b) involving tropine **2** and phenyl acetic acid **3** again yielded no intermediate **4** in the full scan MS in a solvent screen that included acetonitrile (ACN), dichloromethane (DCM), dimethylacetamide (DMA), dimethylformamide (DMF), dimethyl sulfoxide (DMSO), ethanol, methanol, tetrahydrofuran (THF), and toluene. By contrast, the third pathway (scheme 1c), using tropine **2**, phenylacetyl chloride **5** and HCl in dioxane yielded **4** in 30 % conversion (figure 2a). The fourth pathway (scheme 1d) eliminated the use of acid, with only tropine **2** and phenylacetyl chloride **5** in the solvent. A solvent screen using the nine previously used solvents was conducted for this pathway to esterification of tropine **2** with phenylacetyl chloride **5**. The intermediate **4** was produced in DMA, DMF, ethanol and methanol, with the highest percent conversion (55%) being shown in DMA (figure 2b). This pathway to the synthesis of intermediate **5** has not been published in bulk and was first described observed in a preliminary communication on the utilization of this screening methodology.[54] The conversion varied in different permutations of the pathway with the finding that tropine **2** and phenylacetyl chloride **5** in DMA produced the highest conversion of the four pathways. This was a promising pathway to carry forward to microfluidics testing. Because solvents ethanol and methanol are expected to lead to aldol condensation byproducts, only DMA and DMF were examined in microfluidics.

Scheme 1: Routes to intermediate **4**

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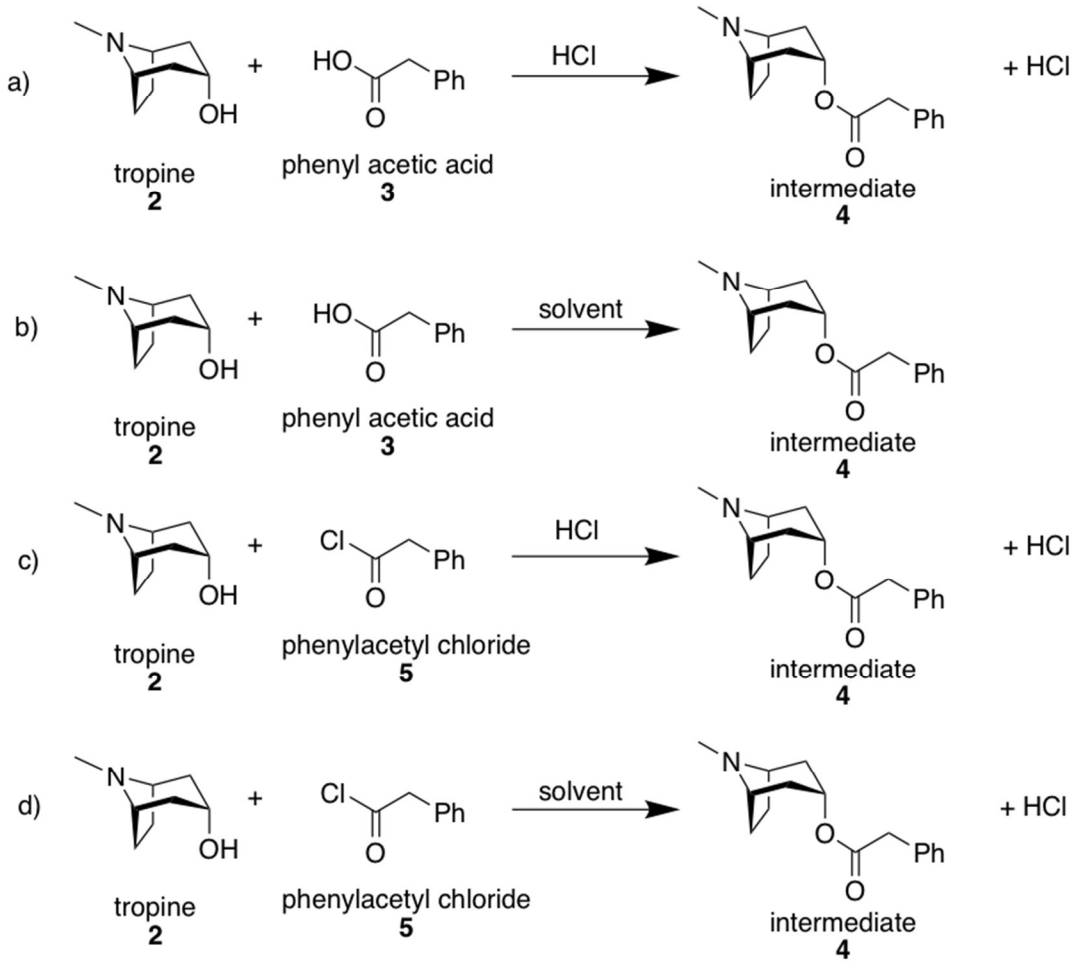

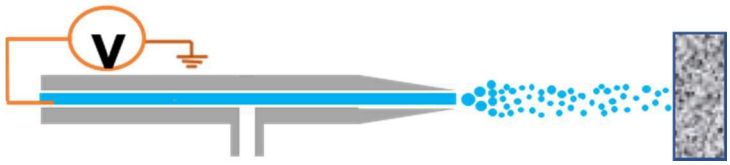

Figure 1: Preparative ES High voltage is applied to the dissolved reaction mixture in a syringe and nitrogen assists in nebulizing the solution. Spray droplets containing product and unconverted reagent are deposited on glass wool for analysis.

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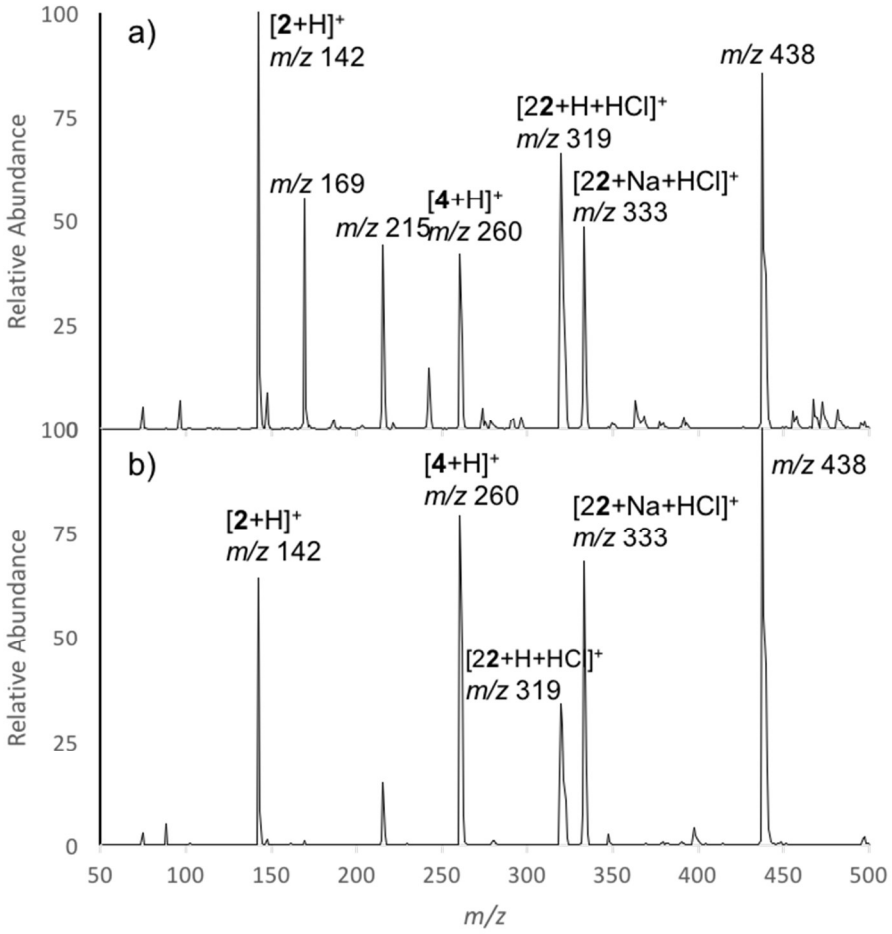

Figure 2: Mass spectra of ES deposited material a) Full scan positive ion mode spectrum of deposited material from preparative ES reaction of tropine **2**, phenylacetyl chloride **5** and HCl in dioxane yielding intermediate **4** (30% conversion). b) Full scan positive ion mode spectrum of the deposited material from the preparative ES reaction of tropine **2**, and phenylacetyl chloride **5** in DMA yielding intermediate **4** (55% conversion). 2**2** indicates the dimer of 2.

Preparation of Intermediate 4 in Flow

The two best pathways (Scheme 1: **c** & **d)** and solvents, DMA and DMF, for the synthesis of the intermediate ester **4** in preparative ES were examined in microfluidics. The flow reaction between tropine **2** (1 equivalent) and phenylacetyl chloride **5** (1.1 equivalent) in the presence of HCl in dioxane in DMA (Scheme 1, **c**) showed 91% conversion as judged by LC-MS of the intermediate salt **4** at 100°C in 9.16 minutes (Supplemental Table 1). The second pathway, used tropine **2** (1 equivalent) and phenylacetyl chloride **5** (1.1 equivalent) (Scheme 1, d) in DMA and

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at 150°C in 4 minutes gave 94% conversion of the intermediate **4** along with quaternary ammonium salt byproducts (Supplemental Scheme 1 and Supplemental Table 2). The quaternary ammonium salts were produced from reaction between the ester intermediate and phenyl acetyl chloride. The presence of solvent associated byproducts was noted at 150°C and 200°C (Supplemental Scheme 2). The microfluidic reaction using DMF as the solvent showed more solvent associated byproduct than did DMA. For neither of these processes was reaction quenching performed.

Reducing the amounts of the quaternary ammonium salts byproducts is important because it decreases the probability of producing further byproducts in the next step of the atropine synthesis. To achieve this equimolar (1:1) amounts of tropine **2** and phenylacetyl chloride **5** were used: this gave 89% conversion of intermediate **4** with less quaternary ammonium byproducts at 100°C in 2 minutes (Supplemental Table 2). The latter route eliminates the step of producing the tropine salt using hydrochloric acid and both routes have similar conversions to intermediate **4**. This route is 3.8 fold faster than the reported flow synthesis of atropine.[49] Preparative ES and microfluidics data both show that the highest conversion pathway to the production of intermediate **4** was tropine **2** and phenylacetyl chloride **5** in DMA.

Synthesis of Atropine by Preparative Electrospray

The second step in the synthesis of atropine is a base-catalyzed aldol condensation with the addition of formaldehyde and base to the intermediate **4**. The crude product from the preparative ES reaction of tropine **2** and phenylaceyl chloride **5** in DMA, was telescoped with aqueous formaldehyde (37%) and 1M base in DMA to form atropine. Twenty-two bases were screened in preparative ES to determine which base yielded the highest conversion to atropine with the least amount of byproduct. This methodology allowed quick screening of different bases to determine which yielded the highest conversion to atropine and simultaneously allowed examination of the byproducts produced by each base. Of the 22 bases screened (Supplemental Table 3), seven showed conversions to atropine of more than 5%. The percent conversion included accounting for byproducts previously identified in the continuous flow synthesis by aldol condensation of atropine and aqueous formaldehyde in both sodium hydroxide and pH 10 buffer. Seven bases

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successfully produced atropine, therefore, the best bases for the aldol condensation were judged by both high conversion to atropine and low conversion to byproducts. The bases 1,8-diazabicyclo[5.4.0]undec-7-ene, potassium ethoxide, potassium methoxide, sodium ethoxide, sodium hydroxide and sodium methoxide had a higher percent conversion to apoatropine **6,** the E1 elimination byproduct, than atropine. The base 1,5-diazabicyclo[4.3.0]non-5-ene was the screened base with the highest conversion (44.5%) to atropine, moreover it was the only base with only nominal production of byproducts. The preparative ES data therefore indicate that the best base for the synthesis of atropine in microfluidics is likely to be 1,5-diazabicyclo[4.3.0]non-5-ene.

Telescoped Synthesis of Atropine in Flow using Two Chips

The hydroxymethylation step (aldol condensation, 2nd step) was examined under different reaction conditions using the intermediate from the first step in microfluidics without any further purification (Figure 2). Nine bases were screened, which encompassed both successful and unsuccessful reactions in preparative ES and their conversions to product are summarized in table 1. Apoatropine **6** (E1 elimination byproduct) was produced under all conditions as E1 elimination is accelerated by temperature and base. Sodium ethoxide in ethanol produced solid during the microfluidic reaction leading to a clogged chip because the byproduct (sodium chloride) has a low solubility in ethanol. The pH 10 buffer led to an overall conversion to atropine of 30% and this condition produced byproducts **6** and **8** (Scheme 2) (Supplemental Table 4). Increasing the temperature led to more elimination byproduct **7**, the higher temperatures driving water elimination from atropine. Increasing the amount of formaldehyde or the residence time increased the second aldol byproduct **8.** Decreasing the temperature did not lead to the production of atropine. Sodium methoxide and potassium methoxide gave 24% overall conversions, again as measured by MS. However increasing residence time and temperature increased the production of byproducts **6** (Supplemental Table 5)**.** A new byproduct, **7,** arose by Michael addition of methanol present in the base as well as in formaldehyde solutions. Trimethylamine and dabco showed poor conversion to atropine, even at higher temperature and longer residence time.

Scheme 2: Possible byproducts from second step reaction

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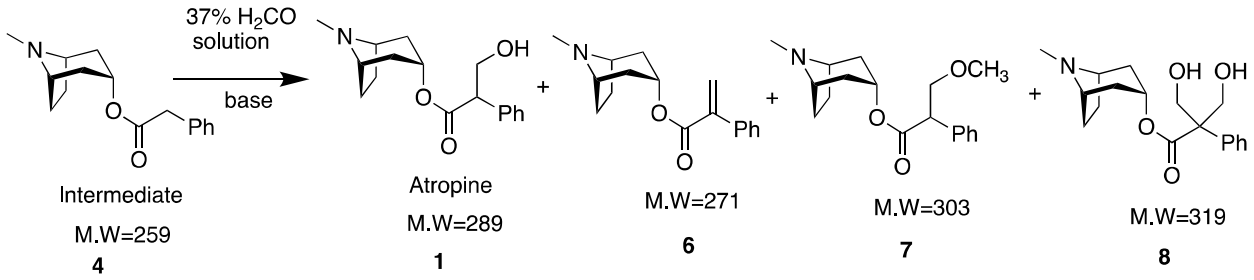

Tetramethylammonium hydroxide took less than a minute to convert all intermediate to product and byproducts. The maximum conversion to atropine (36%) was associated with large amounts of byproducts 6, **7.** The best condition for tetramethylammonium hydroxide with the highest conversion (32%) and the lowest conversion to byproducts was found at 100°C in 8.6-second (Supplemental Table 5). Sodium hydroxide showed 23% maximum overall conversion of atropine in 6-minute residence time with hydrolyzed tropine and byproducts **6, 8** (Supplemental Table 7).

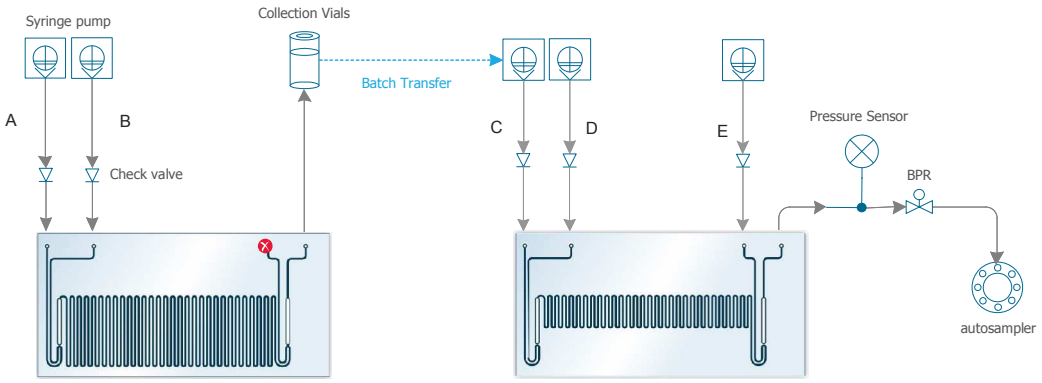

Figure 3: Synthesis of Atropine in flow in two chips A = Tropine; B = Phenylacetyl chloride, C = crude product from first chip; D = Base + Formaldehyde; E = Water

The 1,5-diazabicyclo[4.3.0]non-5-ene was the most effective base for the aldol condensation reaction in microfluidics, which correlates to the preparative ES data. The maximum overall conversion of atropine using 1,5-diazabicyclo[4.3.0]non-5-ene base was 44% (Table 1) at 70°C in 4 and 6 minutes residence time and it showed minimum amounts of byproduct **6**. Increasing residence time and temperature increased byproduct **6**. The maximum atropine yield (not

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conversion) measured quantitatively by NMR was 30 % at 70°C using the 6 minutes residence time.

Table 1: Conversion of crude intermediate 4 to atropine (1) and to byproducts

Base	Maximum conversion of atropine (%)	Reaction conditions	Byproducts in flow	Preparative ES conversion (%)	Byproducts in ES
pH 10 buffer	30	150°C, 2min	**7, 9**	1.6	**7**
Sodium methoxide in methanol (CH_3ONa)	24	150°C, 2.9min	**7,8**	9.9	**7**
Potassium methoxide in methanol (CH_3OK)	24*	70°C, 2min	**7,8**	7.6	**7,9**
Tetramethyl ammonium hydroxide	36*	100°C, 21.4 sec	**7,8**	1.6	**7,9**
Sodium hydroxide	23	70°C, 2min	**7, 9**	15.4	**7**
1,5-Diazabicyclo[4.3.0]non-5-ene	44*	70°C, 6min	**7**	47.5	**7**

Maximum percent conversion of atropine in two steps in two different chips. The percent conversion was determined by nESI-MS unless asterisked * in which cases conversion was determined by LC-MS. The reaction conditions describe the temperature and residence time in the second chip.

The reaction conditions (chip 3227, 4 equiv of formaldehyde, 4 equiv base, 70°C , P = 9 bar) for the highest conversion of 1,5-diazabicyclo[4.3.0]non-5-ene were used to compare the relative conversion of atropine in 1,5-diazabicyclo[4.3.0]non-5-ene, potassium methoxide, sodium hydroxide and pH 10 buffer (Supplemental Table 7). The overall conversion of atropine using 1,5-diazabicyclo[4.3.0]non-5-ene in two steps was almost identical in 4 and 6 minutes. Atropine conversion decreased with increasing residence time for both potassium methoxide and sodium

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hydroxide bases. The pH10 buffer did not lead to the production of atropine in this reaction condition.

The base 1,5-diazabicyclo[4.3.0]non-5-ene gave the smallest conversion to byproducts and showed only 3.8% of byproduct **6** in 6 min residence time (Table 2). The best base for the aldol condensation was 1,5-diazabicyclo[4.3.0]non-5-ene in both preparative ES and microfluidics.

Table 2: Conversion to byproducts in different bases

Byproduct	Method	Percent Conversion of Byproducts (%)		
		1,5-diazabicyclo[4.3.0]non-5-ene	CH_3OK	NaOH
6	ES	11.4	45.1	40.3
	Flow	3.8	20.2	65.8
7	ES	<1	<1	<1
	Flow	<1	44.8	<1
8	ES	2.2	31.1	1.4
	Flow	1.5	4.1	13.4

Telescoped Synthesis of Atropine by Extractive Electrospray

The multi-step synthesis of atropine was performed continuously using a variant on extractive electrospray (EES) (figure 4). This technique utilized two electrospray emitters where the first ESI emitter produced the intermediate **4** and the second ESI emitter nebulized the hydroxymethylation reagents for the second and final step of the atropine synthesis. The reactive EES setup used differs from traditional EES. In traditional EES the angle of intersection of the two ES emitters is 90, with some experiments using angles of 40-60 degrees.[38, 40] For these reactive EES experiments, the angle of intersection is <22.5% which allows for more interactions of the droplets from the ES emitters. This was also favored by increasing the distance of their intersection point to the collecting surface 4 cm. This allows for adequate reaction time for the first step of the reaction to proceed before interacting with droplets from the second step reagents. The setup was optimized using different angles of emitters, distance of the emitters to droplet intersection and droplet intersection to the glass wool, and applied polarities (supplemental figures 30-33). When the ES emitters were orthogonal, as in traditional EES, the ratio of product to intermediate to was low. The highest ratio of product to intermediate occurred

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when the emitters were placed at an angle of ca. 22.5^0. Higher ratios of product to intermediate occurred when the point of intersection of the plume from both emitters and distance from the intersection to the collection surface was greater, however there was a trade off in quantity of material deposited on the glass wool. In preparative reactive EES, the unreacted phenylacetyl chloride reacted with the water in aqueous formaldehyde to form the of phenyl acetic acid seen in MS as the protonated dimer $[2M+H]^+$. This was expected as phenylacetyl chloride is highly reactive with water. Despite this, the percent conversion to atropine was 24% which is similar to the total conversion from both steps in the preparative ES of 26.%. Therefore, telescoped preparative EES is a viable approach to microscale synthesis with similar conversions to telescoped preparative ES.

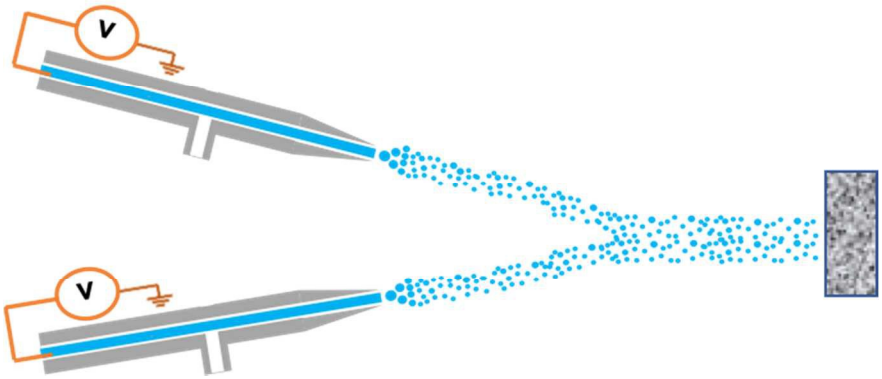

Figure 4: Preparative reactive EES High voltage is applied to the solvent through a platinum electrode and nitrogen gas assists in nebulizing the solution. The ES emitters are positioned at an angle of 22.5^0 and the point of intersection of the spray plumes is 4 cm from the glass wool collection surface.

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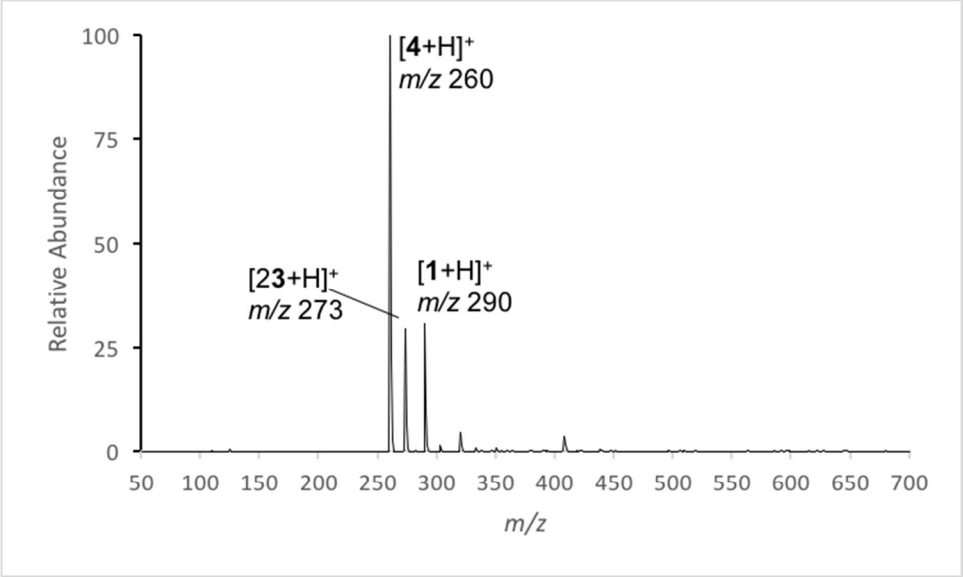

Figure 5: Mass spectrum (positive ion mode) of material deposited using preparative reactive EES showing the atropine product **1** and intermediate **4**.

Continuous Microfluidic Synthesis of Atropine

Telescoping in one reactor 3224 reactor caused more byproduct formation and gave less control over reaction conditions so this encouraged us to telescope the atropine synthesis in two separate reactors. The optimized conditions using 1,5-diazabicyclo[4.3.0]non-5-ene base in the separate steps (1st step: 2min, 100°C and 2nd step: 6 min, 70°C) allowed telescoped atropine synthesis in 55.1% conversion by nanoESI-MS. The average quantified yield was 33.5% by RP-UPLC.

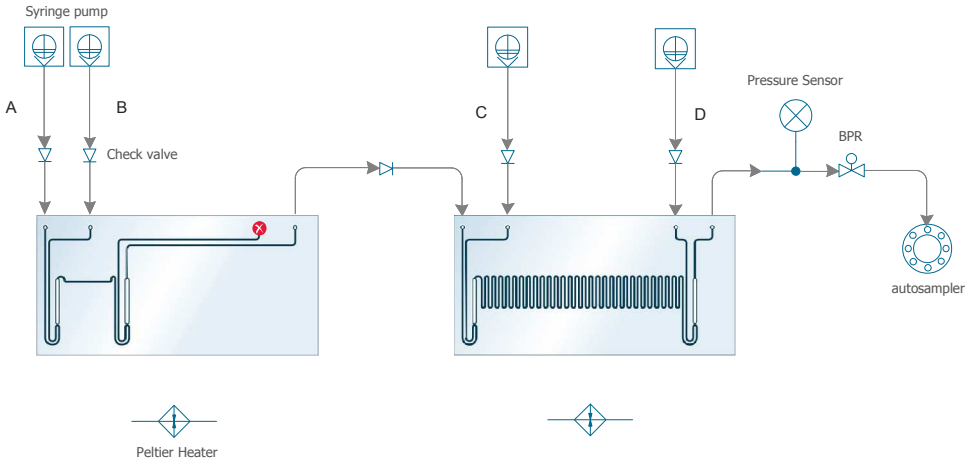

Figure 6: Continuous flow synthesis of atropine in separate reactors

Comparison of Preparative ES and Microfluidics

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Preparative ES is a fast method which successfully predicted the reaction pathway, solvent and base with the highest percent conversion in microfluidics. Naturally, there were noticeable differences between the results of the two techniques in both conversion to products and in production of byproducts. The conversion from starting material to product was somewhat higher in microfluidics than in preparative ES. This can be attributed to the larger scale, the longer time, the greater ability to control reactions in microfluidics and the use of elevated temperatures. Elevated temperatures have been used for preparative ES experiments[54, 55] but the successful synthesis of the intermediate at room temperature showed that heat was unnecessary for the binary reaction screen. However, elevated temperatures may be useful for screening other reactions. Another differentiating factor is the open atmosphere in preparative ES. In microfluidics, the reaction occurs in a closed pressurized system which limits interfaces to glass/solution interfaces unlike the air/solution interfaces in preparative ES. A third factor affecting the difference in conversion is the vapor pressure of both the reagents and solvents. Reaction acceleration in preparative ESI relies heavily on solvent evaporation from the charged droplets[22] Volatile reagents may leave the droplets rather than remain in the (presumably) highly reactive interfacial positions on its surface.

There were differences in the formation of byproducts for the second step of the atropine synthesis in preparative ES and microfluidics. While both techniques heavily favored the E1 eliminated byproduct, **6**, major difference lay in the production of byproducts **7** and **8**. In preparative ES, **7** was not produced and conversion to byproduct **8** occurred to give more than 5% conversion in every successful base with the exception of 1,5-diazabicyclo[4.3.0]non-5-ene, sodium hydroxide and sodium methoxide. Conversely, byproduct **7** was produced in microfluidics with the bases sodium methoxide, potassium methoxide and tetramethyl ammonium hydroxide. The only bases to produce byproduct **8** with conversion greater that 5% were sodium hydroxide and pH 10 buffer. Byproduct **8** is formed when two formaldehyde molecules react with the intermediate **4,** while byproduct **7** is the Michael addition product to methanol to **6** at high temperature. Byproduct **7** was not formed in preparative ES due to the lack of heat necessary for the Michael addition.

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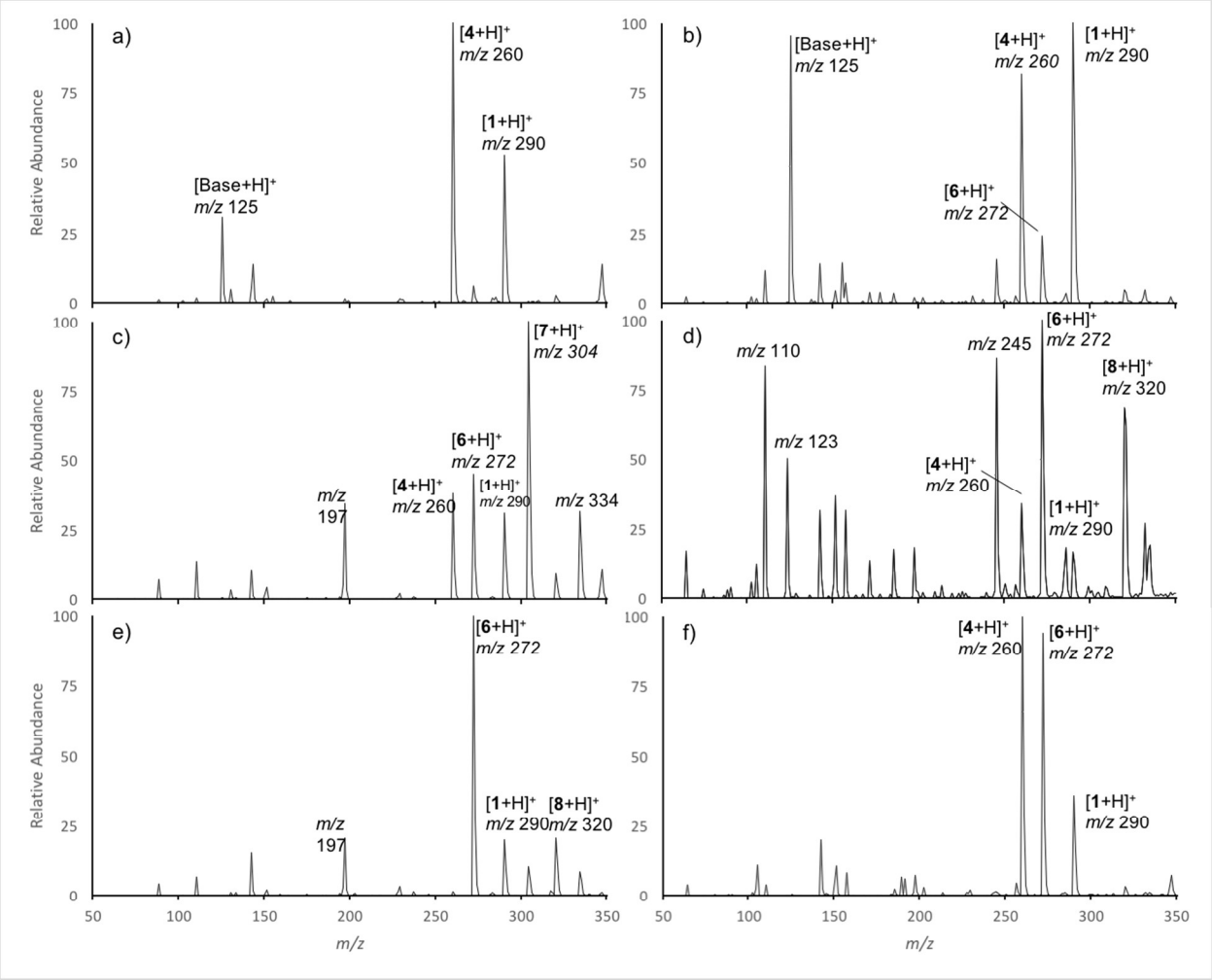

Figure 7: Mass spectra of microfluidic and preparative ES products using three different bases. Full scan positive ion mode spectrum of atropine produced by synthesis using a) 1,5-diazabicyclo[4.3.0]non-5-ene in microfluidics. b) 1,5-diazabicyclo[4.3.0]non-5-ene in preparative ES, c) potassium methoxide in microfluidics, d) potassium methoxide in preparative ES, e) sodium hydroxide in microfluidics, f) sodium hydroxide in preparative ES.

Conclusion

This is the first investigation of spray reactions guiding microfluidic synthesis where preparative ES has been explicitly used for route screening, solvent screening and acid/base screenings on a large scale. Preparative ES was used as a rapid way to discover new synthetic pathways and this methodology led to faster optimization of microfluidic reactions by determining and eliminating unsuccessful reaction pathways from further consideration. Pathways discovery to determine

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new and faster reactions for formulations from raw materials can improve the current manufacturing workflow in the pharmaceutical and chemical industries.

This investigation led to a highly efficient first step esterification of tropine to intermediate without the use of tropine salt or added acid. The first step was optimized in flow utilizing the information obtained in the charged microdroplets. The intermediate from the first step in preparative ES was used to screen 22 unique bases for the base-catalyzed aldol condensation to form the final product, atropine. Seven bases were shown to yield atropine and with the exception of transient solids, all lead to the production of atropine in flow. In both preparative ES and continuous microfluidics the base with the highest conversion and lowest conversion to byproducts was 1,5-diazabicyclo[4.3.0]non-5-ene. In preparative ES the first step conversion to intermediate **4** of 55% and the second step yielded atropine in 47% conversion, with an overall conversion of 26%. In flow, the atropine yield was 30% by NMR (44% conversion by nanoESI-MS). Atropine was continuously synthesized in both preparative EES and microfluidics with conversion of 24% in preparative EES (by nanoESI-MS) and 34% yield in flow (by RP-UPLC). The correlation of the data between preparative ES and microfluidics provides evidence that accelerated reactions in droplets can guide microfluidics.

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Notes and references

1 V. Hessel, D. Kralisch, N. Kockmann, T. Noël, Q. Wang, *ChemSusChem,* 2013, ***6***, 746-789.
2 J.-i. Yoshida, Y. Takahashi, A. Nagaki, *Chemical communications,* 2013, ***49***, 9896-9904.
3 J. Wegner, S. Ceylan, A. Kirschning, *Chem Commun (Camb),* 2011, ***47***, 4583-4592.
4 D. Webb, T. F. Jamison, *Chemical Science,* 2010, ***1***, 675-680.
5 K. Geyer, T. Gustafsson, P. H. Seeberger, *Synlett,* 2009, 2382-2391.
6 K. F. Jensen, *Mrs Bulletin,* 2006, ***31***, 101-107.
7 A. Adamo, R. L. Beingessner, M. Behnam, J. Chen, T. F. Jamison, K. F. Jensen, J. C. Monbaliu, A. S. Myerson, E. M. Revalor, D. R. Snead, T. Stelzer, N. Weeranoppanant, S. Y. Wong, P. Zhang, *Science,* 2016, ***352***, 61-67.
8 D. R. Snead, T. F. Jamison, *Angew Chem Int Ed Engl,* 2015, ***54***, 983-987.
9 D. Kralisch, G. Kreisel, *Chemical engineering science,* 2007, ***62***, 1094-1100.
10 R. L. Hartman, K. F. Jensen, *Lab Chip,* 2009, ***9***, 2495-2507.
11 H. Kim, K. I. Min, K. Inoue, D. J. Im, D. P. Kim, J. Yoshida, *Science,* 2016, ***352***, 691-694.
12 L. Malet-Sanz, F. Susanne, *J Med Chem,* 2012, ***55***, 4062-4098.

View Article Online
DOI: 10.1039/C7AN00622E

13 I. R. Baxendale, R. D. Braatz, B. K. Hodnett, K. F. Jensen, M. D. Johnson, P. Sharratt, J. P. Sherlock, A. J. Florence, *Journal of Pharmaceutical Sciences,* 2015, ***104***, 781-791.
14 T. Gustafsson, F. Ponten, P. H. Seeberger, *Chemical Communications,* 2008, 1100-1102.
15 M. D. Hopkin, I. R. Baxendale, S. V. Ley, *Org Biomol Chem,* 2013, ***11***, 1822-1839.
16 B. J. Deadman, M. D. Hopkin, I. R. Baxendale, S. V. Ley, *Organic & Biomolecular Chemistry,* 2013, ***11***, 1766-1800.
17 P. Zhang, M. G. Russell, T. F. Jamison, *Organic Process Research & Development,* 2014, ***18***, 1567-1570.
18 D. R. Snead, T. F. Jamison, *Chemical Science,* 2013, ***4***, 2822-2827.
19 P. R. D. Murray, D. L. Browne, J. C. Pastre, C. Butters, D. Guthrie, S. V. Ley, *Organic Process Research & Development,* 2013, ***17***, 1192-1208.
20 X. Yan, E. Sokol, X. Li, G. Li, S. Xu, R. G. Cooks, *Angewandte Chemie International Edition,* 2014, ***53***, 5931-5935.
21 J. J. Agresti, E. Antipov, A. R. Abate, K. Ahn, A. C. Rowat, J.-C. Baret, M. Marquez, A. M. Klibanov, A. D. Griffiths, D. A. Weitz, *Proceedings of the National Academy of Sciences,* 2010, ***107***, 4004-4009.
22 R. D. Espy, M. Wleklinski, X. Yan, R. G. Cooks, *TrAC Trends in Analytical Chemistry,* 2014, ***57***, 135-146.
23 M. Girod, E. Moyano, D. I. Campbell, R. G. Cooks, *Chemical Science,* 2011, ***2***, 501-510.
24 G. Xu, B. Chen, B. Guo, D. He, S. Yao, *Analyst,* 2011, ***136***, 2385-2390.
25 A. K. Badu-Tawiah, L. S. Eberlin, Z. Ouyang, R. G. Cooks, *Annual review of physical chemistry,* 2013, ***64***, 481-505.
26 J. Laskin, P. A. Eckert, P. J. Roach, B. S. Heath, S. A. Nizkorodov, A. Laskin, *Analytical chemistry,* 2012, ***84***, 7179-7187.
27 R. M. Bain, C. J. Pulliam, S. A. Raab, R. G. Cooks, *Journal of Chemical Education,* 2015, ***93***, 340-344.
28 R. M. Bain, C. J. Pulliam, R. G. Cooks, *Chemical Science,* 2015, ***6***, 397-401.
29 E. A. Crawford, C. Esen, D. A. Volmer, *Analytical Chemistry,* 2016, ***88***, 8396-8403.
30 D. Schröder, *Accounts of chemical research,* 2012, ***45***, 1521-1532.
31 R. H. Perry, T. J. Cahill, J. L. Roizen, J. Du Bois, R. N. Zare, *Proceedings of the National Academy of Sciences,* 2012, ***109***, 18295-18299.
32 D. N. Mortensen, E. R. Williams, *Journal of the American Chemical Society,* 2016, ***138***, 3453-3460.
33 R. H. Perry, M. Splendore, A. Chien, N. K. Davis, R. N. Zare, *Angewandte Chemie,* 2011, ***123***, 264-268.
34 R. H. Perry, K. R. Brownell, K. Chingin, T. J. Cahill, R. M. Waymouth, R. N. Zare, *Proceedings of the National Academy of Sciences,* 2012, ***109***, 2246-2250.
35 T. Müller, A. Badu-Tawiah, R. G. Cooks, *Angewandte Chemie International Edition,* 2012, ***51***, 11832-11835.
36 S. Banerjee, E. Gnanamani, X. Yan, R. N. Zare, *Analyst,* 2017,
37 H. Chen, A. Venter, R. G. Cooks, *Chemical Communications,* 2006, 2042-2044.
38 L. Zhu, G. Gamez, H. W. Chen, H. X. Huang, K. Chingin, R. Zenobi, *Rapid Communications in Mass Spectrometry,* 2008, ***22***, 2993-2998.

View Article Online
DOI: 10.1039/C7AN00622E

39 K. Chingin, G. Gamez, H. Chen, L. Zhu, R. Zenobi, *Rapid Communications in Mass Spectrometry,* 2008, ***22***, 2009-2014.
40 H. Chen, S. Yang, M. Li, B. Hu, J. Li, J. Wang, *Angewandte Chemie International Edition,* 2010, ***49***, 3053-3056.
41 H. Chen, R. Zenobi, *Nature protocols,* 2008, ***3***, 1467-1475.
42 H. Chen, A. Wortmann, R. Zenobi, *Journal of Mass Spectrometry,* 2007, ***42***, 1123-1135.
43 H. Chen, A. Wortmann, W. Zhang, R. Zenobi, *Angewandte Chemie International Edition,* 2007, ***46***, 580-583.
44 L. Zhu, G. Gamez, H. Chen, K. Chingin, R. Zenobi, *Chemical Communications,* 2009, 559-561.
45 J. K. Lee, S. Kim, H. G. Nam, R. N. Zare, *Proceedings of the National Academy of Sciences,* 2015, ***112***, 3898-3903.
46 R. Qiu, C. Zhang, Z. Qin, H. Luo, *RSC Advances,* 2016, ***6***, 36615-36622.
47 G. Grynkiewicz, M. Gadzikowska, *Pharmacol Rep,* 2008, ***60***, 439-463.
48 M. Gadzikowska, G. Grynkiewicz, *Acta Pol Pharm,* 2002, ***59***, 149-160.
49 C. H. Dai, D. R. Snead, P. Zhang, T. F. Jamison, *Journal of Flow Chemistry,* 2015, ***5***, 133-138.
50 R. Robinson, *Journal of the Chemical Society, Transactions,* 1917, ***111***, 762-768.
51 G. C. Schmidt, T. E. Eling, J. C. Drach, *Journal of Pharmaceutical Sciences,* 1967, ***56***, 215-221.
52 J. R. Pfister, *The Journal of Organic Chemistry,* 1978, ***43***, 4373-4374.
53 A.-C. Bédard, A. R. Longstreet, J. Britton, Y. Wang, H. Moriguchi, R. W. Hicklin, W. H. Green, T. F. Jamison, *Bioorganic & Medicinal Chemistry,*
54 M. Wleklinski, C. E. Falcone, B. P. Loren, Z. Jaman, K. Iyer, H. S. Ewan, S. H. Hyun, D. H. Thompson, R. G. Cooks, *European Journal of Organic Chemistry,* 2016, ***2016***, 5480-5484.
55 H. Chen, L. S. Eberlin, M. Nefliu, R. Augusti, R. G. Cooks, *Angewandte Chemie International Edition,* 2008, ***47***, 3422-3425.

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Reaction mixtures are screened by droplet ES synthesis; optimum reagents and conditions as determined by MS analysis then are transferred to the micrrofluidic system where the screened reagents are retested and conditions are further optimized. This rapid screening/optimization approach is demonstrated for the synthesis of atropine.