Supporting Information

Segmented Flow Platform for On-Demand Medicinal Chemistry and Compound Synthesis in Oscillating Droplets

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System Setup

Overall control: Control and automation of the system is written entirely in LabVIEW 2014 and MATLAB R2015a. Droplets are represented by custom classes to take advantage of Matlab's object-oriented programming capabilities: each droplet object contains information regarding its composition, current position, online injection volume, intended reaction time, actual reaction time, along with various other attributes and flags (e.g., whether the droplet is in the system).

Droplet preparation: Droplets are prepared by a Gilson GX-271 liquid handler according to reaction conditions defined in an Excel spreadsheet document. First, a 20 μL inert gas buffer is aspirated to prevent excessive contact between reagents and the carrier fluid in the liquid handler line. The make-up solvent and each reagent are then aspirated from vials stored in the liquid handler vial rack in turn according to the target composition and stock solution concentrations, with external needle rinsing between each one. The total prepared volume of 35 μL is oscillated inside the liquid handler needle repeatedly to pre-mix the droplet and ensure homogeneity. The droplet is delivered to the flow system, consisting of clear FEP tubing (primarily 1/16" O.D., 0.02" I.D.), via a Gilson Direct Injection Module and a 14 μL sample loop. Because the droplet is prepared at atmospheric pressure and the system is pressurized, it is imperative that the sample loop in the Direction Injection module is fully liquid-filled before switching the valve to the system position.

Droplet motion: Motion of the droplet in the oscillatory flow chemistry platform is controlled by a PHD Ultra syringe pump (Harvard Apparatus) which infuses/withdraws the 100 psig argon carrier gas. After preparation and injection into the sample loop, droplets are sent downstream towards the reactor by infusing the carrier gas syringe (8 mL stainless steel gastight). The carrier syringe is automatically refilled once its fill volume becomes too low, approximately every three reaction slugs.

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‡ Electronic Supplementary Information (ESI) available: System set-up, synthesis in batch, HPLC chromatograms, NMR spectra, calibration curves, characterization of on-line injection. See DOI: 10.1039/x0xx00000x

Injection timing: While droplet position can be estimated by integrating the known carrier flow rate with respect to time, this approach does not afford the required level of spatial accuracy for online injection into an already-flowing droplet. Off-the-shelf optical sensors (OPB350 series, Optek Technology) are used to detect the presence or absence of liquid within the transparent tubing; each sensors' signal is read into LabVIEW using a USB-6001 data acquisition device (National Instruments). Combined with knowledge of the sensors' positions relative to T-junctions, this produces an extremely reliable trigger for online injections into the already-flowing droplet (**Figure S14**). Injections themselves are carried out by PHD 2000 syringe pumps.

Reactor control: After an optional online injection, the slug is moved to a single-point oscillatory flow reactor (SPOFR). The custom-fabricated aluminum reactor houses 1/8" O.D. clear FEP tubing arranged so that the inlet and outlet are in parallel. One LED and one photodetector (Thorlabs, Inc.) are attached on opposite sides of these two tube segments so that a slug passing by the inlet or outlet triggers a change in photodetector voltage. By combining this voltage information with the slug position tracked in Matlab, the droplet can be reliably kept oscillating inside the reactor for the target residence time (via repeated reversal of carrier gas flow direction). The temperature of the reactor is monitored and controlled by an Omega temperature controller and two cartridge heaters (Watlow, 50W each); reaction temperature is limited by the choice of tubing (for fluoroethylpropylene (FEP), ~210 Celsius). After each reaction step, according to the user-defined conditions, the slug is sent (a) to the online injection T-junction for an additional injection before returning to the reactor, (b) to the outlet injection for an additional reagent injection before returning to the reactor, or (c) to the outlet injection for a quench injection before continuing downstream.

Analysis: The quenched droplet passes through an 8 μL loop in a Gilson 6-port 2-way valve. Upon detecting liquid (via another phase sensor) immediately downstream of the valve, the position is switched so that the sample loop is then in the flow path of an Agilent HPLC/MS/ELSD, bypassing that unit's own liquid handler. A remote trigger is sent to Agilent ChemStation to begin the analysis method. The whole 8 μL sample is sent through an analytical or semi-prep column and multi-wavelength detector (MWD). An active splitter separates a small fraction (1/50) to be sent to the MS/ELSD for destructive analysis, while the remainder moves through a delay loop. If specified in ChemStation, the UV and/or MS signals are used to trigger fraction collection of the isolated product. The full analysis results are exported so a spreadsheet which can then be read directly in LabVIEW. An image of the analysis units is shown in **Figure S15**.

User interface: Details of the stock solutions loaded into a reagent block are defined in an Excel spreadsheet. Also in this spreadsheet is a list of reaction conditions, which are defined by compound numbers (unique tags corresponding to the stock solutions), target concentrations, make-up solvent, reaction temperatures, reaction times, and any additional injection information required for multi-step chemistry. All system parameters (e.g., reactor volume) are defined on the LabVIEW front panel. The system is configured to be monitored and controlled remotely and is frequently operated through a Remote Desktop connection.

Quantification using ELSD: All products were able to be collected by utilizing the fraction collector triggered either by target mass or target retention time. Convenient quantification by

ELSD allows direct use of the synthesized compounds to be tested in biological or physicochemical assays. ELSD is known to show an exponential relationship of $A = aP^b$, where A is a peak area and P is a mass of product. The calibration curve as shown in **Figure S12** was obtained with a various standards of N-(4-methoxyphenyl)-2-phenylacetamide in acetonitrile, which is the product from the acylation of substrates **c** and **7**. Calculated constants a and b were 1.05429 and 1.08254, respectively, with an R square value of 0.97556 by origin curve fitting of 7 data points.

To investigate the accuracy of quantification by ELSD, the yield estimated by ELSD should be compared to isolated product mass. Because the screening platform is designed to synthesize sub-miligram quantities of compounds on demand, product from multiple runs must be combined to produce a sufficient quantity to weigh. An acylation between 1-phenylpiperazine (**b**) and pyrazinecarboxylic acid (**8**) was performed 12 times, and product was isolated from each run. Superimposed HPLC chromatogram for all 12 runs is shown in **Figure S13**. The chromatogram was almost identical for 12 runs with an averaged conversion of 68.3 % and standard deviation of 1.0%, confirming the excellent reproducibility of our system. The average estimated mass of product calculated from ELSD measurement was 76.6 ± 4.1 mg, and this mass was compared to the product mass of calculated based on the conversion (117.3 ± 1.8 mg). We note that the conversion was considered as the yield with no significant side reaction in this acylation. There was a large 34.7% difference although this magnitude of error in ELSD quantification is not inconsistent with other reported values (~ 30%). Product quantification of sulfonylation and S_NAr reaction of 1-phenylpiperazine (**b**) were also tested with substrates **5** and **3**, and the difference of product mass estimated from ELSD and calculated conversion was 13.0% and 16.5%, respectively. This level of uncertainty, however, is in an acceptable range for preliminary analysis of the drug lead via biological or physicochemical assays.

ELSD Parameters: Evaporation temperature (75 ˚C), nebulizer temperature (85 ˚C), gas flow (2.0 SLM), LED intensity (100%), smoothing (50), PMT gain (10).

Suzuki-Miyaura Coupling Reaction in batch

Synthesis of 2-phenylpyridine. 2-Chloropyridine (28.8 mg, 0.254 mmol) and phenylboronic acid (45.73 mg, 0.375 mmol) with XPhos Pd G3 (4.24 mg, 0.005 mmol) were added into a 5 mL oven-dried volumetric flask equipped with a magnetic stir bar and fitted with a Teflon septum. The vessel was then degassed and filled with argon three times. Afterwards, 1 mL of anhydrous tetrahydrofuran (THF) and 1 mL of 1M K_3PO_4 aqueous solution was added and degassed one at a time. The reaction mixture was stirred at 1000 rpm at 65 ˚C under argon. The samples collected by syringe after 90, 180, 300, 600, and 900 seconds of reaction were cooled down to room temperature in ambient condition, quenched with 1:1 acetone:DI water, extracted with toluene, and analyzed by HPLC.

Synthesis of 2-(3-thenyl)pyridine. 2-Chloropyridine (28.80 mg, 0.254 mmol) and 3-thenylboronic acid (47.99 mg, 0.375 mmol) with XPhos Pd G3 (4.24 mg, 0.005 mmol) were added into a 5 mL oven-dried volumetric flask equipped with a magnetic stir bar and fitted with a Teflon septum. The vessel was then degassed and filled with argon three times. Afterwards, 1 mL of anhydrous tetrahydrofuran (THF) and 1 mL of 1M K_3PO_4 aqueous solution was added and degassed one time. The reaction mixture was stirred at 1000 rpm at 65 ˚C under argon. The samples collected by syringe after 300, 600, 900, and 1200 seconds of reaction were cooled

down to room temperature in ambient condition, quenched with 1:1 acetone:DI water, extracted with toluene, and analyzed by HPLC.

Diclofenac Synthesis in Batch

2-(2-((2,6-Dichlorophenyl)amino)phenyl)acetic acid (diclofenac). 2,6-dichloroaniline (8.10 mg, 0.05 mmol), Sphos Pd G4 (1.98 mg, 0.0025 mmol), NaOtBu (6.73 mg, 0.07 mmol), and internal standard naphthalene were added into a 5 mL oven-dried volumetric flask equipped with a magnetic stir bar and fitted with a Teflon septum. Then, tert-butyl 2-(2-bromophenyl)acetate (16.27 mg, 0.06 mmol) was added, and the vessel was degassed and filled with nitrogen two times. Afterward, 1 mL of anhydrous toluene was added and the vessel was degassed and filled with nitrogen two times. The reaction mixture was stirred at 500 rpm at 90 ˚C under nitrogen for 20 minutes. After the reaction mixture is cooled down to 40 ˚C, acetonitrile with 0.1 vol % formic acid was added and stirred for 10 minutes. The crude reaction mixture was analyzed by HPLC. The product and intermediate were purified by using silica gel thin layer chromatography (TLC) glass plates, and confirmed by MS and ^{1}H NMR.

Results

	Amine	Aryl halide	Solvent	Temp	Time
Original	benzylamine (0.16 M)	2-fluoropyridine (0.04 M)	NMP	140 C	2700 s
		(rinsing routine)			
Blank	none	none	NMP	140 C	300 s

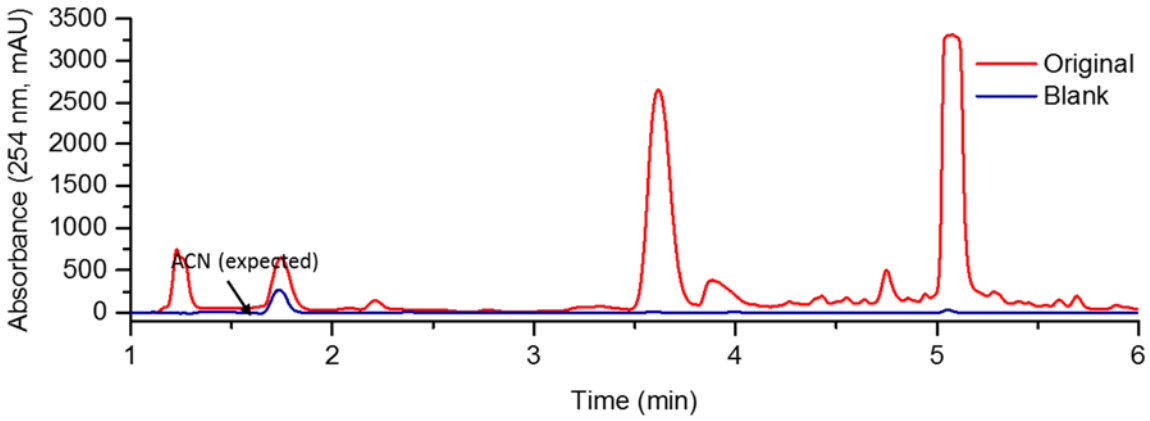

Fig. S1. Carryover test showing chromatograms for a substitution reaction between benzylamine and 2-fluoropyridine in NMP (original) and a blank slug of NMP (blank) following the standard rinsing routine. After each reaction, the autosampler needle is dipped into clean solvent twice; approximately 160 uL of clean solvent is flushed through the needle into the sample loop to clean the injection port; fifteen 15-20 uL rinse slugs of clean solvent are injected upstream of the injection port and flow through the system to clean the tubing. The area of the biphenyl peak at 5.1 minutes for the blank slug is < 0.5% the area for the original slug.

In NMP	Amine	Aryl halide	Temp	Time	Collection
a	1-phenylpiperazine (0.01 M)	4-chloroquinazoline (0.005 M)	140 C	600 s	MS (291.2)
b	benzylamine (0.08 M)	2-chlorobenzoxazole (0.08 M)	80 C	600 s	MS (225.1)
c	phenethylamine (0.08 M)	2-chlorobenzoxazole (0.08 M)	80 C	600 s	MS (239.1)
d	1-phenylpiperazine (0.08 M)	2-chlorobenzoxazole (0.08 M)	80 C	600 s	MS (280.1)
e	benzylamine (0.16 M)	2-fluoropyridine (0.04 M)	140 C	1800 s	Time + UV
f	phenethylamine (0.08 M)	2-fluoropyridine (0.04 M)	140 C	1800 s	Time + UV

Fig. S2. Sequence conditions for six distinct reactions with different reagents, temperatures, times, and collection methods. The sequence was run twice for a total of 12 reactions.

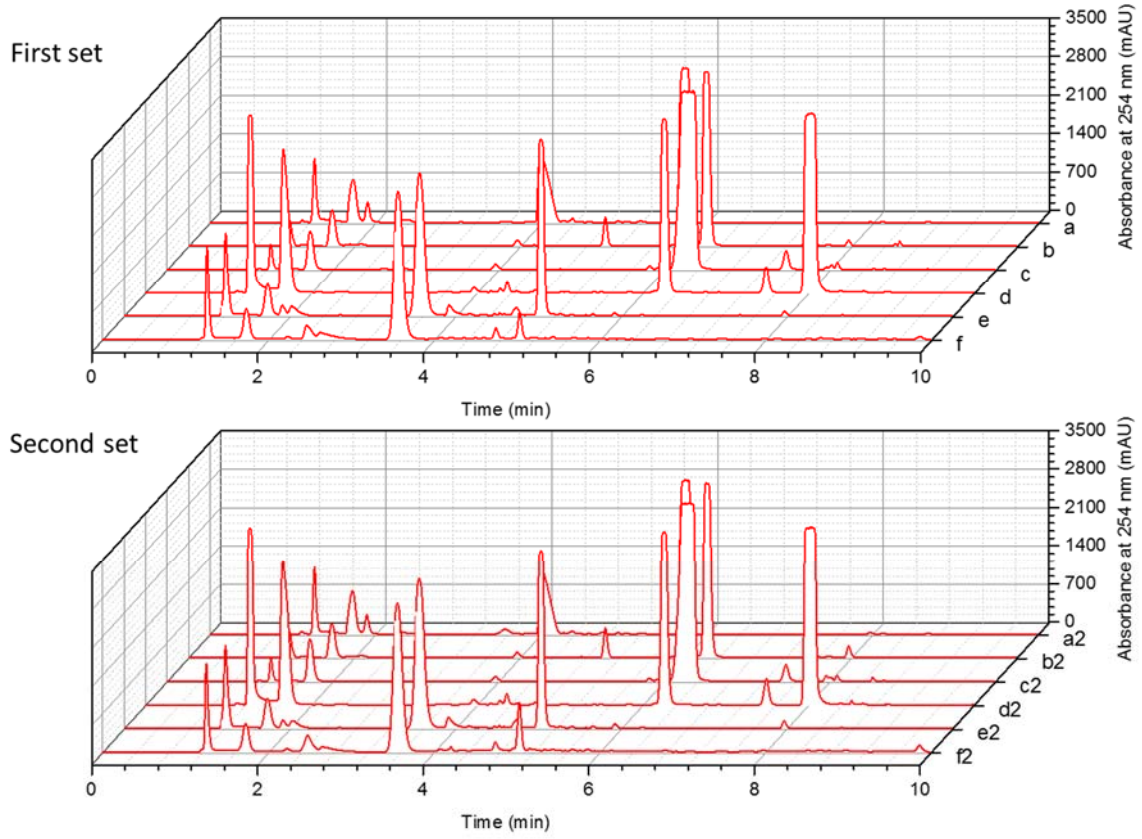

Fig. S3. Stacked chromatograms showing the high reproducibility of the six-reaction sequence.

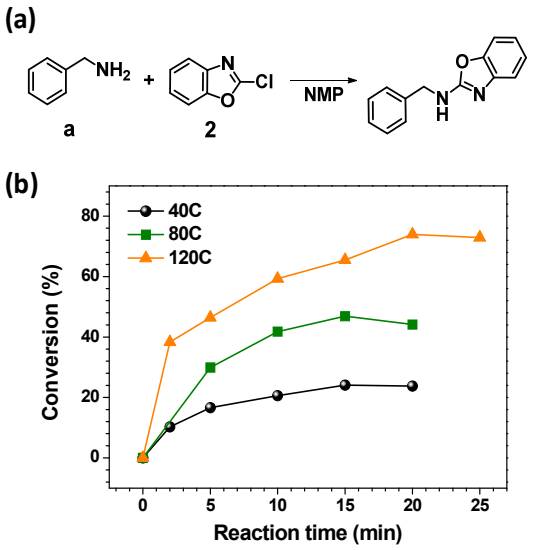

Fig. S4. (a) Synthetic scheme of aromatic substitution (S_NAr) reaction between benzylamine and 2-chlorobenzooxazole. (b) Resulting conversions as a function of reaction time for different reaction temperatures (40, 80 and 120 ˚C).

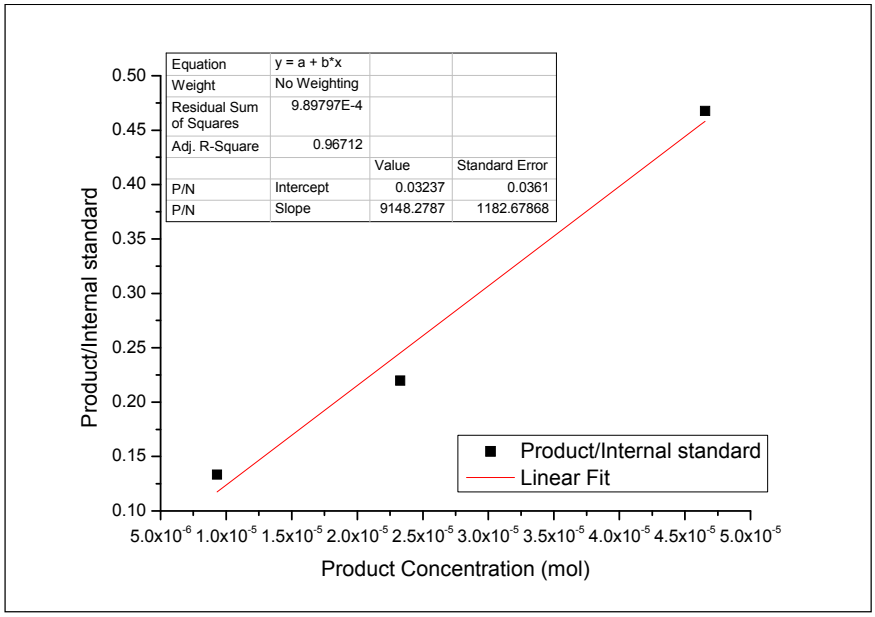

Fig. S5. Calibration curve for the yield calculation in acylation.

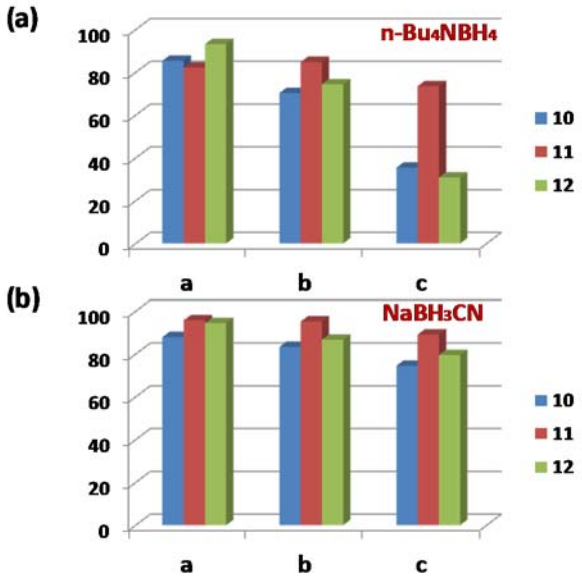

Fig. S6. Conversions of reductive aminations with n-Bu_4NBH_4 (a) and $NaBH_3CN$ (b) as a reducing reagent for the nine pair-wise combinations of amines **a**, **b**, and **c** and aldehydes **10**, **11**, and **12**.

Fig. S7. Modified reactor design for multi-phase reaction. In order to achieve organic-aqueous phase separation during each oscillation pass, it is necessary to have a high cross-sectional velocity; because the carrier fluid is compressible, there is a delay between changing syringe direction and affecting droplet motion. Extending the length of the reactor by 4 cm after the optical detection point prevents the droplet from exiting the reactor during this delay. Additionally, in the multi-phase configuration, a higher flow rate is used when switching directions (e.g., 1500 uL/min) before switching back to the target flow rate (e.g., 750 uL/min) to decrease the delay between the reversal of syringe pump direction and the reversal of droplet motion. The aluminium chuck is further extended after the optical detection point to ensure that the slug remains at the desired reaction temperature even with partial overshooting.

Table S1. Summary of yield (%) of the Suzuki-Miyaura cross-coupling reactions in flow and in batch.

Residence time (s)	Reaction 1 (OFR)	Reaction 1 (Batch)	Residence time (s)	Reaction 2 (OFR)	Reaction 2 (Batch)
0	0	0	0	0	0
90	36.5	33.2	300	76.1	62.2
180	77.9	57.5	600	85.4	87.1
300	86.2	72.5	900	93.6	92.2
600	106.5	94.1	1200	96.2	102.7
900	107.0	107.2			

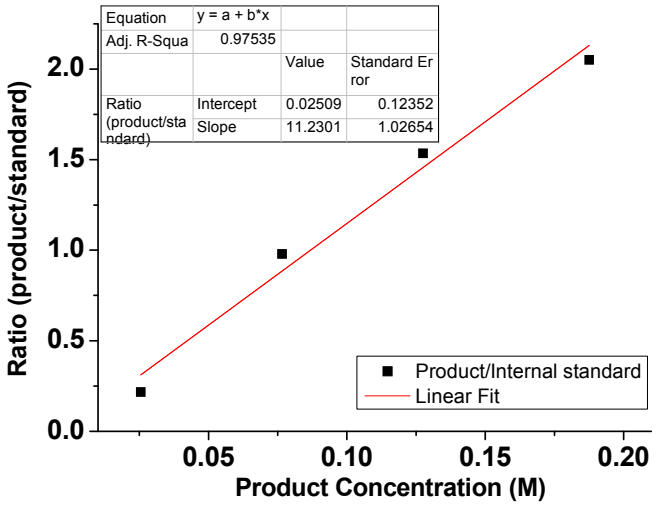

Fig. S8. Calibration curve for the yield calculation in Suzuki-Miyaura cross coupling reaction.

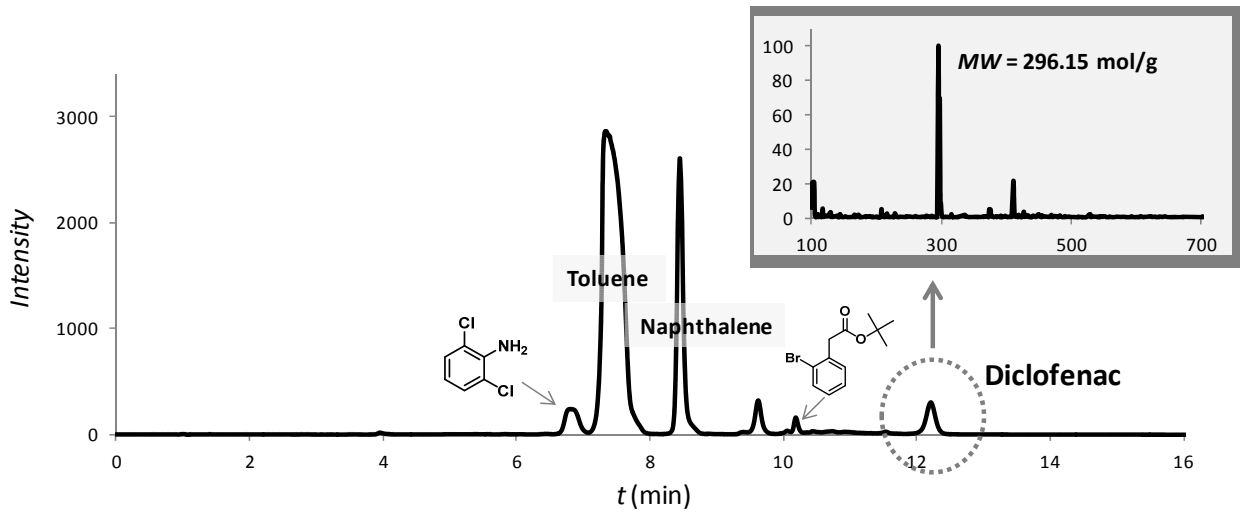

Fig. S9. Resulted HPLC chromatogram of 2-step diclofenac synthesis and imbedded ionized fraction of diclofenac.

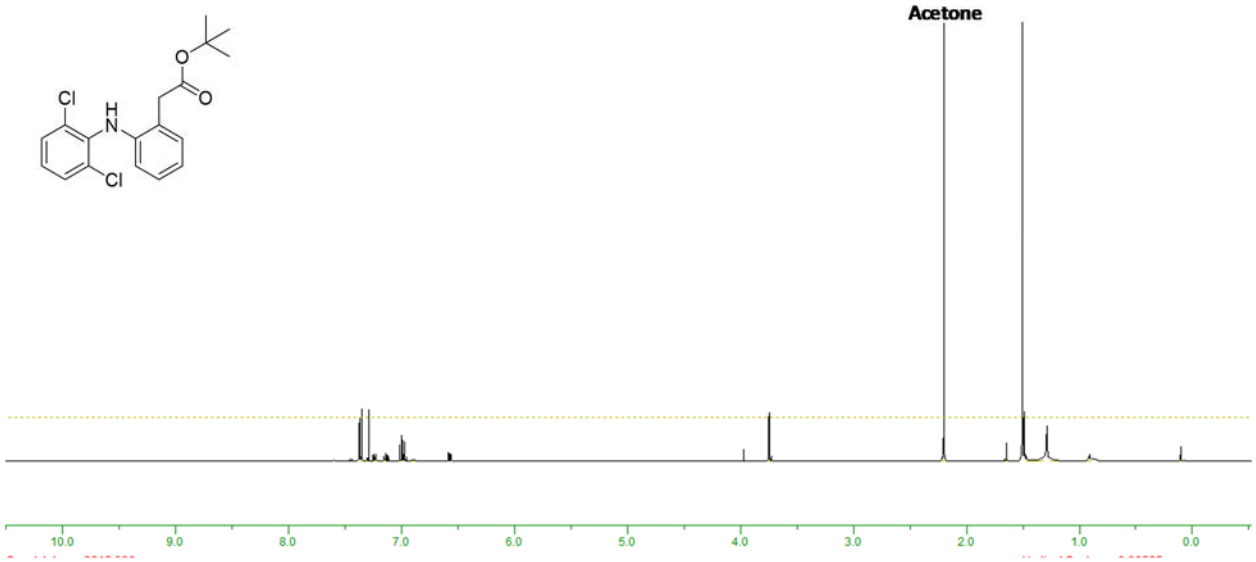

Fig. S10. ^{1}H NMR spectrum of the 1st step product, t-butyl 2-(2-((2,6-dichlorophenyl)amino)phenyl)acetate. ^{1}H NMR spectrum was recorded at 400 MHz in Chloroform-D on a Bruker Advance-400 spectrometer.

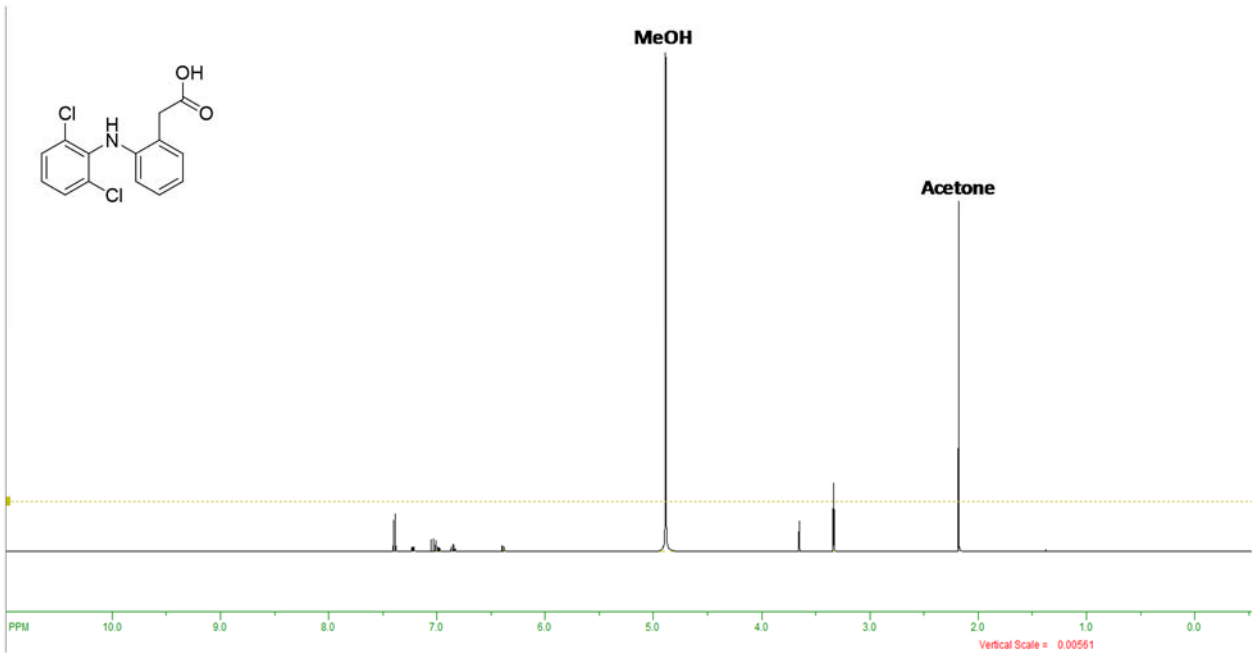

Fig. S11. ^{1}H NMR spectrum of the 2nd step product, 2-(2-((2,6-dichlorophenyl)amino)phenyl)acetic acid (diclofenac). ^{1}H NMR spectrum was recorded at 400 MHz in Methanol-D on a Bruker Advance-400 spectrometer.

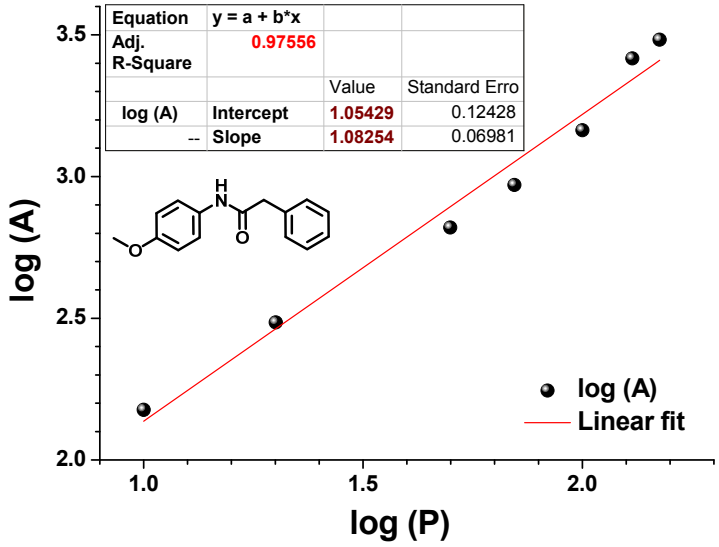

Fig. S12. ELSD calibration curve.

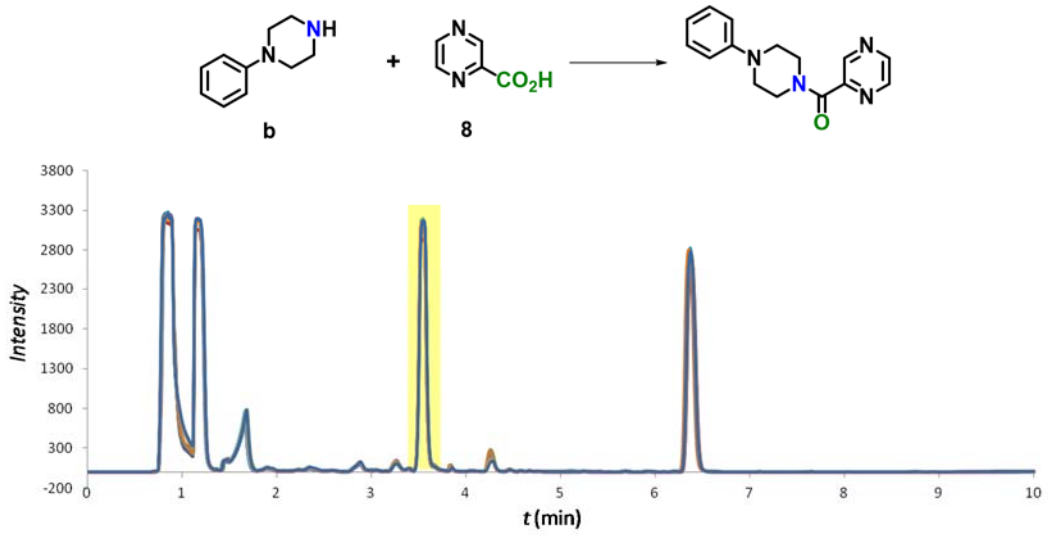

Fig. S13. Overlayed HPLC chromatogram of 12 repeated runs of an acylation reaction between **b** and **8**. The highlighted region corresponds to the product.

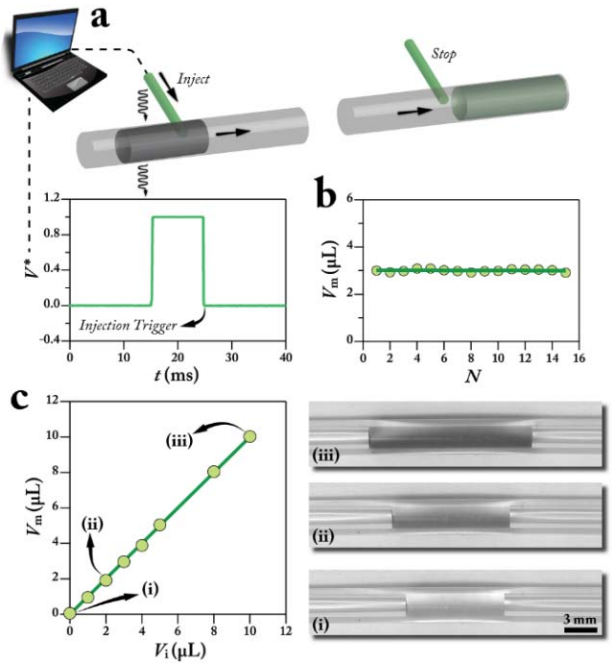

Fig. S14. Characterization of single/multiple on-line injection into the already prepared reagent droplet. (a) use of a phase sensor voltage signal V^* to correctly time injection into an already-flowing droplet. (b) optically-measured injected volumes V_m for 15 repeated 3 μL injections. (c) optically-measured injected volumes V_m compared to intended injection volumes V_i.

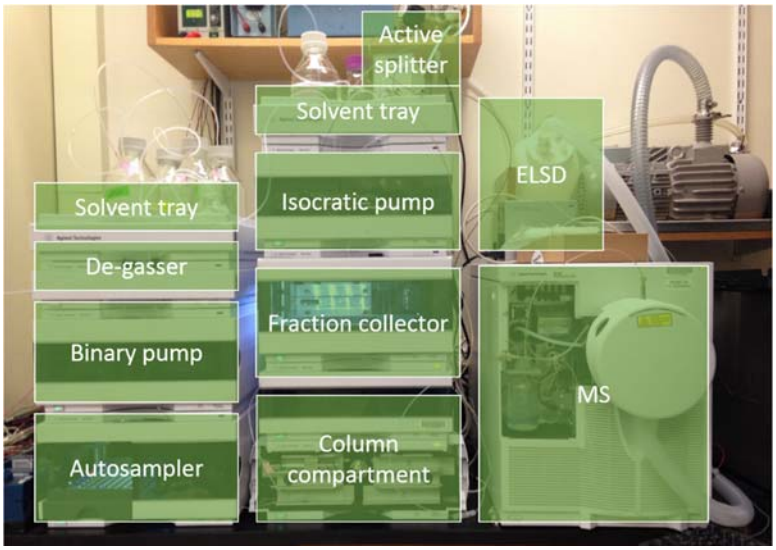

Fig. S15. HPLC/MS/ELSD combined analysis configuration (ELSD not pictured). All components were purchased from Agilent Technologies. The horizontal width of the overall unit is 110 cm (43 inches).

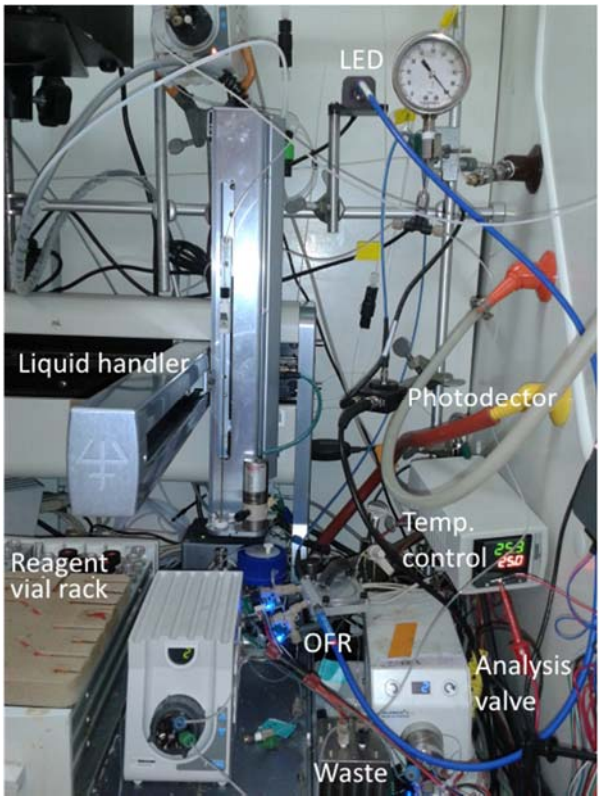

Fig. S16. Reactor configuration. The platform (including pumps to the left, not shown) occupies roughly two-thirds of a standard laboratory fume hood. The system footprint is dominated by the liquid handler.