Electronic Supplementary Material (ESI) for Lab on a Chip.

Supporting Information

A droplet-chip / mass spectrometry approach to study organic synthesis at nanoliter scale

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1. Chip fabrication

For the master structure we used a common photolithographic technique. To get a photomask, the channel structure was designed with Inkscape (Figure S1a) and transferred to a foil by DTP-System-Studio GmbH. A 4-inch silicon wafer was cleaned with an air plasma. After the cleaning step the negative photoresist SU-8 2050 was spin-coated onto the silicon wafer (step 1: 10 s at 500 rpm, step 2: 30s at 2000 rpm; acceleration 300 rpm/s). Subsequently, the silicon wafer with the photoresist was baked (soft bake) (step 1: 3 min at 65 °C, step 2: 9 min at 95 °C). We placed the photomask on top of the wafer. Afterwards the SU-8 2050 layer was illuminated for 15.4 s with the FE5 flood exposure illuminator. For complete curing the wafer with the photoresist was baked (hard bake) (step 1: 2 min at 65 °C, step 2: 7 min at 95 °C). To remove the redundant photoresist, the wafer was washed for 7 min with the developer mr-Dev 600. The SU-8 2050 structure has a height of 80 µm. The microfluidic PDMS (polydimethylsiloxane) device was made by a common softlithographic technique[1]. A commercial available PDMS kit with base and curing agent was mixed in a ratio of 10:1 (w/w). The pre-polymer solution was degassed and applied to the silicon wafer with the SU-8 2050 master structure. After heat induced curing for 1 h at 75 °C, the structured PDMS slide was peeled off, cut and fluidic inlets were stamped. To close the structure a PDMS slice with a height of 200 µm was used. The surface of the two PDMS pieces were activated with an air plasma and afterwards they were pressed together to get a complete bonding. A third PDMS slice with an embedded Teflon tubing was bonded to the chip as a bottom plate. It is important to make sure that the Teflon capillary is below the channel of the steel capillary (Figure S1 a, X4). Afterwards, the chip was cut open and a steel capillary pushed into the main channel outlet (X4). Finally, the inlet tubings were glued onto the chip and the steel capillary was fixed to the Teflon tubing with Teflon tape (Figure S1c). Before use, the chip was flushed with rain-X and isopropanol to get a uniform hydrophobic surface. A photograph of a representative microfluidic device is shown in Figure S1 b/c.

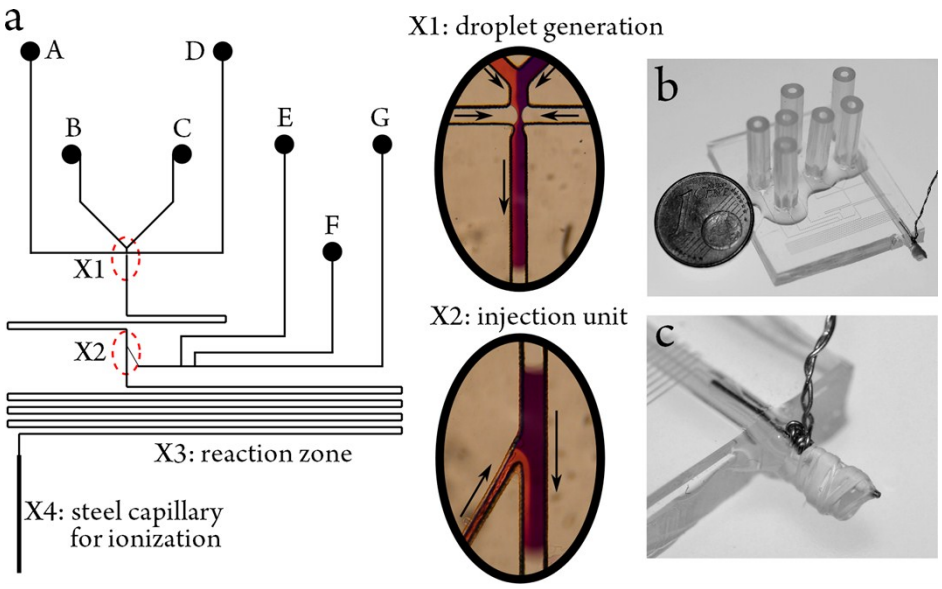


Fig. S1. Chip design with annotated inlets (A-G), functionalities (X1-X4) and two light microscopic images of the droplet generation and the injection unit (a). For a better visualization a purple (crystal violet) and an orange (new coccine) dye were mixed. Photograph of a chip (b) and the enlarged view of the stainless steel capillary and the Teflon tube (c).

2. Experimental procedure

2.1. Coupling of the microfluidic device to mass spectrometry

To ionize the droplets, we used a steel capillary at the chip outlet as ESI emitter. The Teflon capillary works as an oil drain. The microfluidic device is located in front of a metal plate as counter electrode. To get an electrospray, 2.6 to 2.9 kV applied at the tip and 0 kV at the counter electrode. After the removal of the commercial ESI source this construction was placed perpendicular in front of the MS (mass spectrometer) inlet (Figure S2).

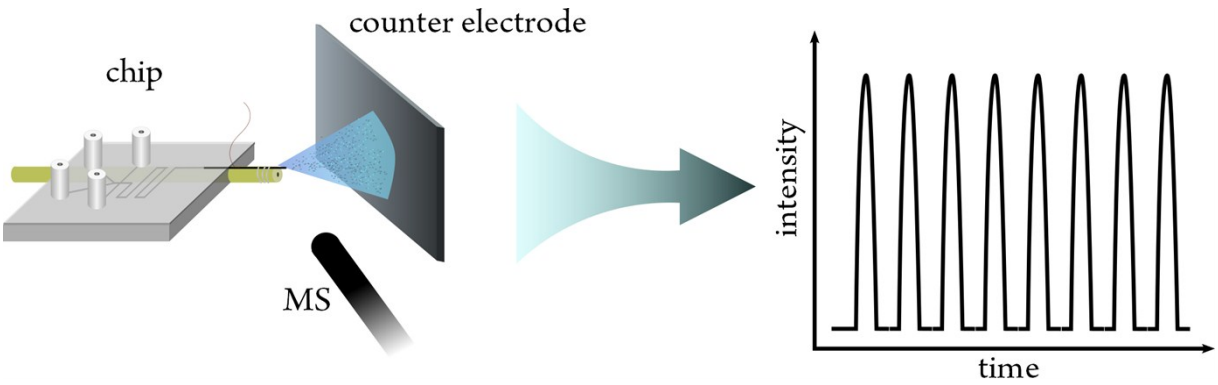


Fig. S2. Scheme of droplet-MS coupling with a counter electrode (left) and schematic illustration of the resulting TIC (right).

The MS parameters for all experiments are listed in the following Table S1.

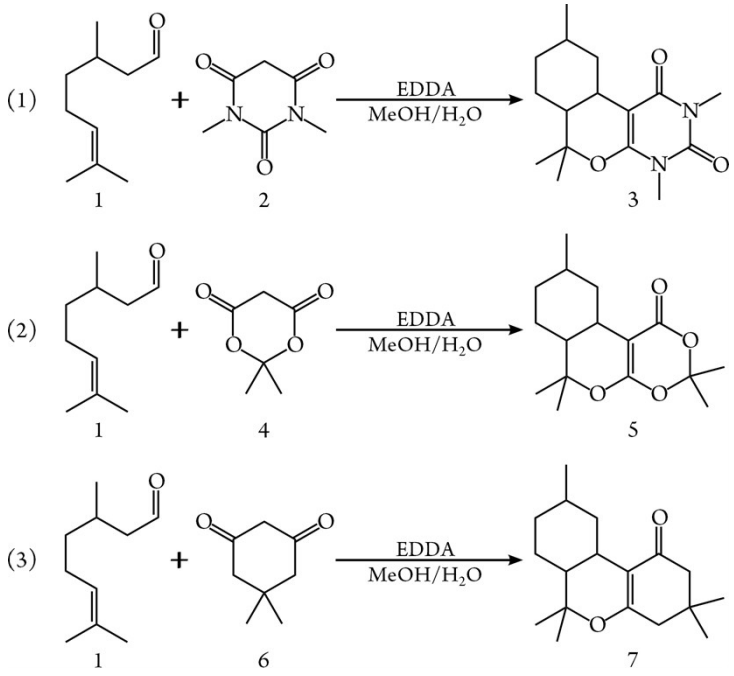
Table S1. Mass spectrometer settings.

Parameter	Setting
Acquisition mode	Positive ion mode, Scan
Mass range	100 – 300 (chapter 2.4 – 2.6); 150 – 250 (chapter 2.3)
Event time	0.05 sec (chapter 2.4 – 2.6); 0.025 (chapter 2.3)
Scan speed	6000 am/sec
Nebulizing gas (N_2)	none

2.2. Domino Knoevenagel-hetero-Diels-Alder reaction

For the on-chip examinations of chemical transformations different domino reactions were used as model systems. On example is a Knoevenagel condensation which is catalyzed by ethylenediammonium diacetate (EDDA). The subsequent step is an intramolecular hetero-Diels – Alder reaction. In Scheme S1 the respective model reactions are given.

Scheme S1. Domino Knoevenagel Hetero-Diels-Alder reactions.



2.3. Location-independent spray stability

To show the location-independent spray stability, we test the microfluidic device with a caffeine solution for the droplets phase and perfluorodecalin as an oil phase. The distance between the emitter tip and the counter electrode was 4 mm and the spacing between the tip and the MS inlet *x* was varied in 100 µm steps. 2.7 kV were applied at the steel capillary and 0 V at the counter electrode.

Table 2: Experimental data for the distance measurements.

Inlet	Substance	Concentration (inside syringe)	Solvent composition	Flow rate
A & D	perfluorodecalin	pure	-	0.2 µL/min
B	caffeine	2 mM	methanol/water (8:2)	0.2 µL/min
C	solvent	pure	methanol/water (8:2)	0.2 µL/min
E, F, G	locked	-	-	-

2.4. Proving basic functionalities of the microfluidic chip device

To verify the correct operation of all functionalities on the microfluidic device, we first investigated reaction 1 (Scheme S1). Droplets filled with a mixture of citronellal (**1**) and barbituric acid (**2**) are generated and analyzed with the seamless hyphenation to electrospray mass spectrometry. After a certain time the catalyst EDDA was injected into each droplet at a downstream dosing functionality to initiate the reaction. To start the injection of EDDA, the flow rate at the inlet E was changed from 0 µL/min to 0.3 µL/min. All substances, concentrations and flow rates are listed in Table S3. Methanol/water (80:20, vol%) is used as the reaction

solvent and perfluorodecalin (PFD) as the continuous carrier phase. 2.9 kV were applied at the steel capillary and the reaction time was 1 min. The assignment of the inlets is shown in Figure S3.

Table S3. Experimental data for the evaluation of the microfluidic device.

Inlet	Substance	Concentration (inside syringe)	Solvent composition	Flow rate
A & D	perfluorodecalin	pure	-	0.2 µL/min
B	citronellal (1)	8.3 mM	methanol/water (8:2)	0.3 µL/min
C	1,3-dimethylbarbituric acid (2)	10 mM	methanol/water (8:2)	0.3 µL/min
E	EDDA	0.83 mM	methanol/water (8:2)	0.3 µL/min or 0 µL/min

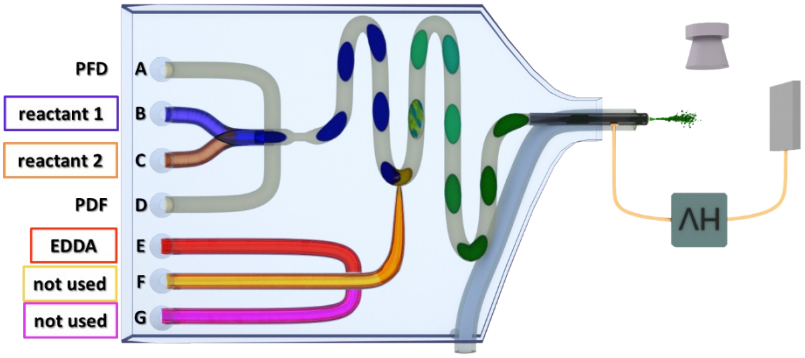


Fig. S3. Schematic representation of the chip and the assignment of the inlets for the evaluation of the microfluidic device.

2.5. Solvent screening

The effect of the solvent compositions on droplet formation was studied in light microscopic experiments. For that purpose a red dyed (New Coccine) methanol/water (50:50, vol%) and a blue dyed (Malachite green) methanol/water (90:10, vol%) solution were mixed on chip. The perfluorodecalin flow rate always was 0.2 µL/min and the sum of the flow rate of the disperse phase was 0.4 µL/min. Representative light microscopy images are shown in figure S4.

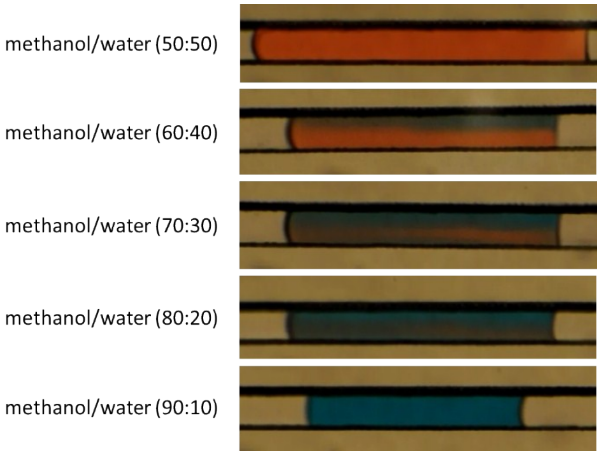


Fig. S4: Light microscopy images of droplets with different solvent compositions. The solutions were dyed with New Coccine (red) and Malachite green (blue).

Different methanol/water compositions were used as solvents for model reaction 1 (Scheme S1). For this purpose the inlets B and C were both filled with reactant **1** and the catalyst with differing the solvent composition (B methanol/water, 90:10, vol%; C:

methanol/water, 50:50, vol%). In an analogous way reactant **2** was introduced via inlets E and F. By adjusting the flow rates a solvent gradient was generated. To get a defined solvent composition the flow rate ratio at B/C and E/F were always the same. Droplets were formed at the droplet generator and the reaction starts immediately after the dosing unit. PFD was used as continuous phase, reaction time was 1 min and 2.6 kV were applied at the steel capillary. All substances, concentrations, solvent compositions and flow rates are listed in Table S4. The assignment of the inlets is shown in Figure S4.

Table S4. Experimental data for the solvent screening.

Inlet	Substance	Concentration (inside syringe)	Solvent composition	Flow rate
A & D	perfluorodecalin	pure	-	0.2 µL/min
B	citronellal (1), EDDA	9 mM (1), 0.9 mM (EDDA)	methanol/water (9:1)	0.4 µL/min → 0 µL/min
C	citronellal (1), EDDA	9 mM (1), 0.9 mM (EDDA)	methanol/water (1:1)	0 µL/min → 0.4 µL/min
E	1,3-dimethylbarbituric acid (2)	12 mM	methanol/water (9:1)	0.3 µL/min → 0 µL/min
F	1,3-dimethylbarbituric acid (2)	12 mM	methanol/water (1:1)	0 µL/min → 0.3 µL/min

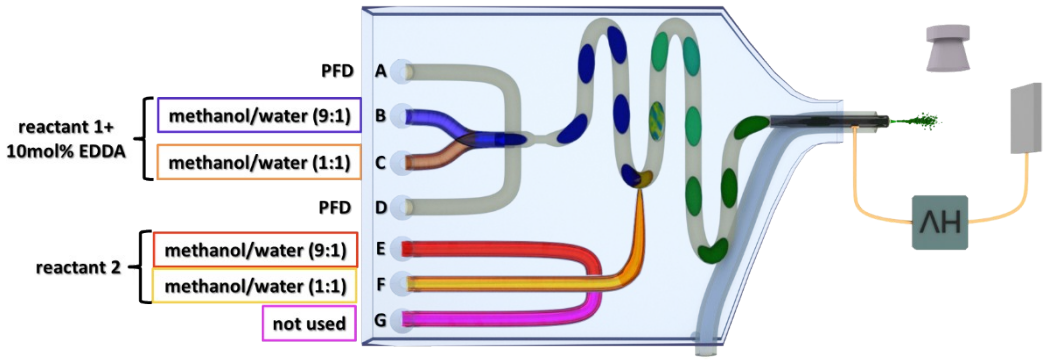


Fig. S5. Schematic representation of the chip and the assignment of the inlets for the solvent screening.

For data evaluation, we used the signal ratio (P/SM) of the product **3** (m/z 293) to the starting material **2** (m/z 157). To consider the influence of the solvent composition on the ionization, we determined a correction factor. Therefore, a mixture of barbituric acid (**2**) (4 mM) and product **3** (0.25 mM) in different solvent compositions was infused by a syringe pump to a conventional ESI source and was analyzed with MS. Fig. S5 shows the normalized signal ratio P/SM in dependence of the water content of the solvent. From these data a correction factor for the chip experiments was determined (Table S5).

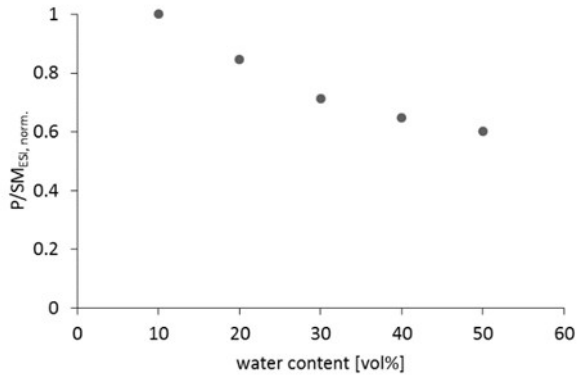


Figure S6: The P/SM ratio of 4 mM barbituric acid (**2**) and 0.25 mM product **3** mixture in methanol/water determine with conventional infusion ESI.

Table S5. Summary of the results of the solvent screening.

Water content [%]	P/SM	Corr. Factor	$P/SM_{corr.}$	$P/SM_{norm., corr.}$	Standard deviation
10	3.393	0.601	2.039	0.236	0.044
20	4.664	0.710	3.314	0.384	0.073
30	7.453	0.843	6.280	0.728	0.143
40	9.308	0.927	8.626	1.000	0.173
50	7.727	1	7.727	0.896	0.186

At first glance, we claim that with more than 40% no further improvement can be observed. To confirm this statement, a statistical hypothesis test (t-test) with the data of 40 % and 30 % water (n=20) was done. The resultant p-value of $3.833*10^{-6}$ show that the difference between these data points is significant. Consequently, the statement was confirmed.

2.6. Examination of a multiple sets of process parameters within a single chip experiment

Three different 1,3-dicarbonyl compounds were examined as starting materials for the reaction with citronellal (**1**). Simultaneously, the concentration of the catalyst was changed. Inlets B and C both introduced reactant **1**, but they differ in the catalyst concentration (B with 0.09 mM EDDA and C with 1.17 mM EDDA). By adjusting the flow rates at these inlets a gradient of EDDA was generated. Droplets with citronellal (**1**) and a defined concentration of EDDA were built at the droplet generator. The reaction starts immediately after the injection of the 1,3-dicarbonyl compound at the downstream dosing unit. The inlets E-G are filled with barbituric acid (**2**), meldrum´s acid (**4**) and dimedone (**6**). Only one of these inlets was active with a flow rate of 0.3 µL/min. By chancing the active channel, we injected different starting materials into the droplets. 2.6 kV were applied at the steel capillary. Table S7 shows all substances, concentrations and flow rates. The assignment of the inlets is shown in Fig. S6.

Table S6. Experimental data for the starting material and catalyst screening.

Inlet	Substance	Concentration (inside syringe)	Solvent composition	Flow rate
A & D	perfluorodecalin	pure	-	0.2 µL/min
B	citronellal (1), EDDA	9 mM (1), 0.09 mM (EDDA)	methanol/water (8:2)	0.4 µL/min → 0 µL/min
C	citronellal (1), EDDA	9 mM (1), 1.17 mM (EDDA)	methanol/water (8:2)	0 µL/min → 0.4 µL/min
E	1,3-dimethylbarbituric acid (2)	12 mM	methanol/water (8:2)	0.3 µL/min or 0 µL/min
F	meldrum's acid (4)	12 mM	methanol/water (8:2)	0.3 µL/min or 0 µL/min
G	dimedone (6)	12 mM	methanol/water (8:2)	0.3 µL/min or 0 µL/min

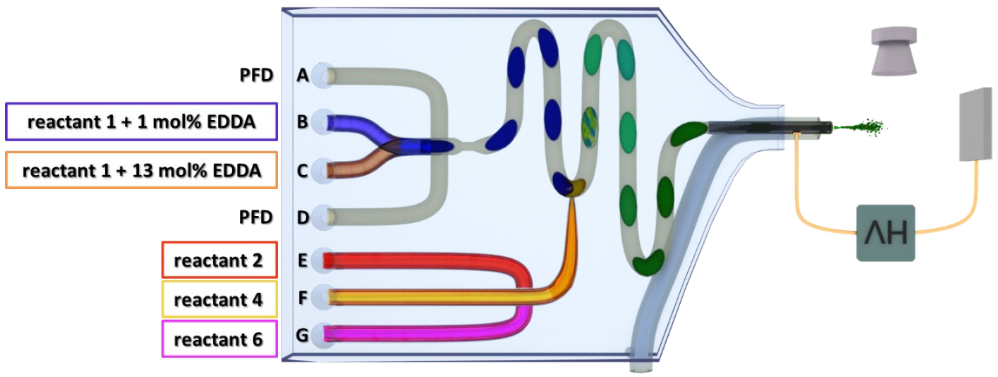


Fig. S7. Schematic representation of the chip and the assignment of the inlets for the starting material and catalyst screening.

[1] D. C. Duffy, J. C. McDonald, O. J. A. Schueller, G. M. Whitesides *Anal. Chem.* **1998**, *70*, 4974–4984.