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Supporting Information

A microdroplet-accelerated Biginelli reaction: mechanisms and separation of isomers using IMS-MS

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General procedure using a theta tip:[1]

The Biginelli reaction was performed by combining an equimolar mixture of urea, benzaldehyde and ethyl acetoacetate in 1:1 MeOH:H_2O. Stock solutions of urea, benzaldehyde and ethyl acetoacetate each with a concentration of 1mM was prepared. This was later diluted to working concentrations of 0.1μM, 10μM, 30 μM, 60 μM and 100 μM. The electrospray ionization was performed using double barrel theta glass capillaries (Warner Instruments, LLC, Hamden, CT), with outer diameter of 1.5mm. This capillary was pulled to a narrow emitter tip (~5 μm) using a model P-97 Flaming/ Brown micropipette puller equipped with an FB330B square box filament (Sutter Instruments Co., Novato, CA), retaining the borosilicate "theta" division. Urea and ethyl acetoacetate were mixed in one channel of the theta glass emitter and benzaldehyde in the other channel. Platinum wires were brought into contact with the solutions in each channel, and electrospray was initiated by applying a voltage of 4.5 kV. Mass spectrometry (MS) and ion mobility spectrometry (IMS)-MS analysis were performed using a LTQ-Velos (Thermo Scientific, San Jose, California) and a home-built 2m IMS-TOF instrument respectively.[2, 3]

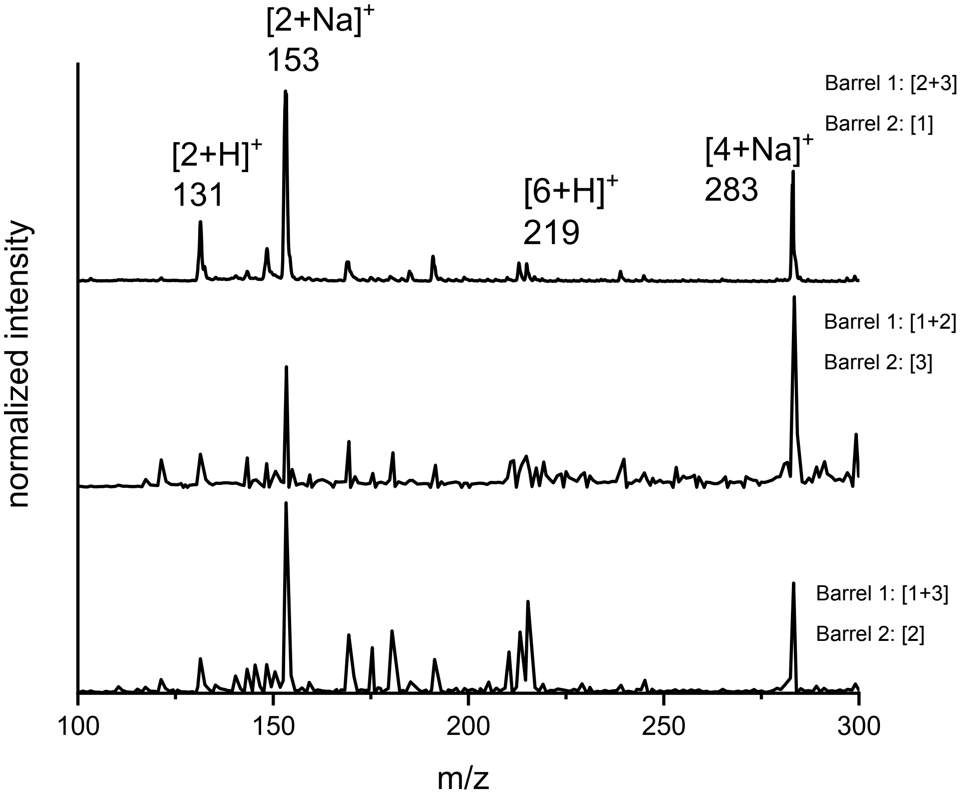


Figure S1. Stack plot of different combinations of reagents spray using theta double-barrel tips.

General procedure using a single-barrel tip:[4, 5]

Equimolar reaction mixtures of urea, ethyl acetoacetate and benzaldehyde were prepared in 1:1 MeOH:H_2O. This was loaded into the single channel tip that was pulled from the borosilicate capillary with outer diameter of 1.5mm using same instrument as mentioned above. Characterization of the inner diameter of this emitter tip was determined to be ~6.1 µm as analyzed using scanning electron microscopy. A single platinum wire was brought in contact with the solution and used to apply an electrical potential of ~4.5 kV, generating the electrospray. The microdroplets formed upon ionization were directed towards the MS-inlet and analyzed.

Bulk-phase preparations:

<u>Bulk-phase preparation of dihydropyrimidinone (**4**):</u>

Compound (**4)** was prepared according to a literature procedure.[6] In a 3-dram vial equipped with a magnetic stir bar, benzaldehyde (0.63 mL, 6.25 mmol), ethyl acetoacetate (1.2 mL, 9.5 mmol), and urea (376.2 mg, 6.25 mmol) were dissolved in alcohol (2.5 mL). HCl (3 drops) was added to the mixture that was then refluxed for 4 hours at 85 °C. A precipitate formed, which was then filtered and washed with cold alcohol to give **(4)**, (1.25g, 77%, in EtOH) and (1.11 g, 69%, in MeOH).

<u>^{1}H NMR:</u> (400 MHz, DMSO-d_6) δ 9.18 (d, *J* = 2.0 Hz, 1H), 7.85 – 7.50 (m, 1H), 7.36 – 7.28 (m, 2H), 7.28 – 7.19 (m, 3H), 5.14 (d, *J* = 3.3 Hz, 1H), 3.98 (q, *J* = 7.1 Hz, 2H), 2.25 (s, 3H), 1.09 (t, *J* = 7.1 Hz, 3H).

13C NMR: (100 MHz, DMSO) δ 165.30, 152.10, 148.33, 144.84, 128.36, 127.23, 126.22, 99.23, 59.16, 53.94, 17.76, 14.06.

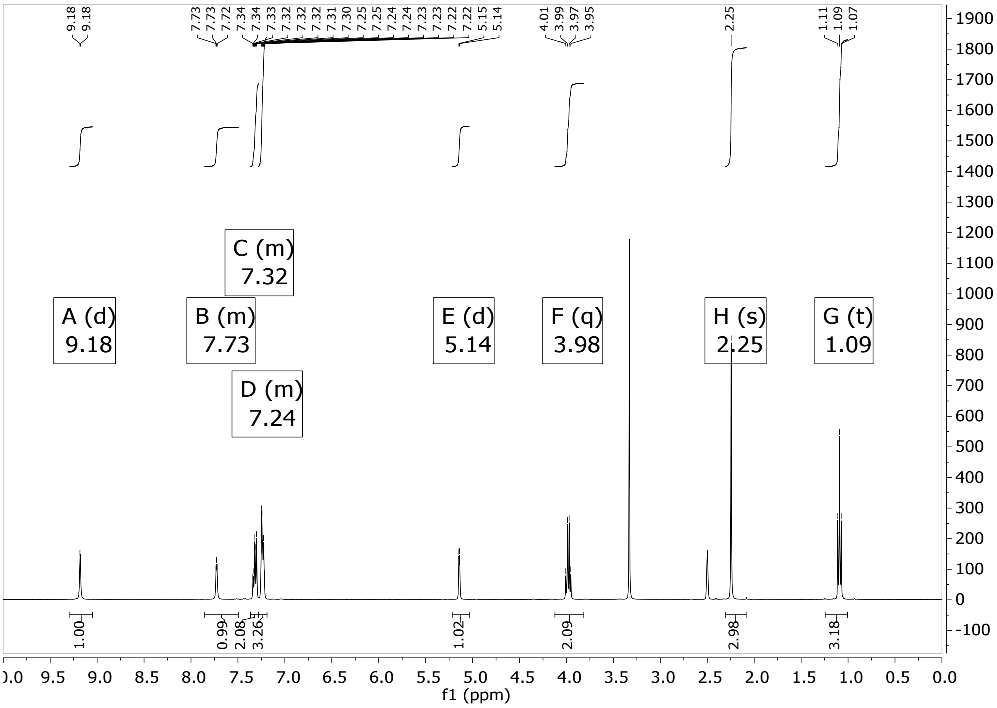


*Figure S2.400 MHz ^{1}H NMR spectrum of (**4**).*

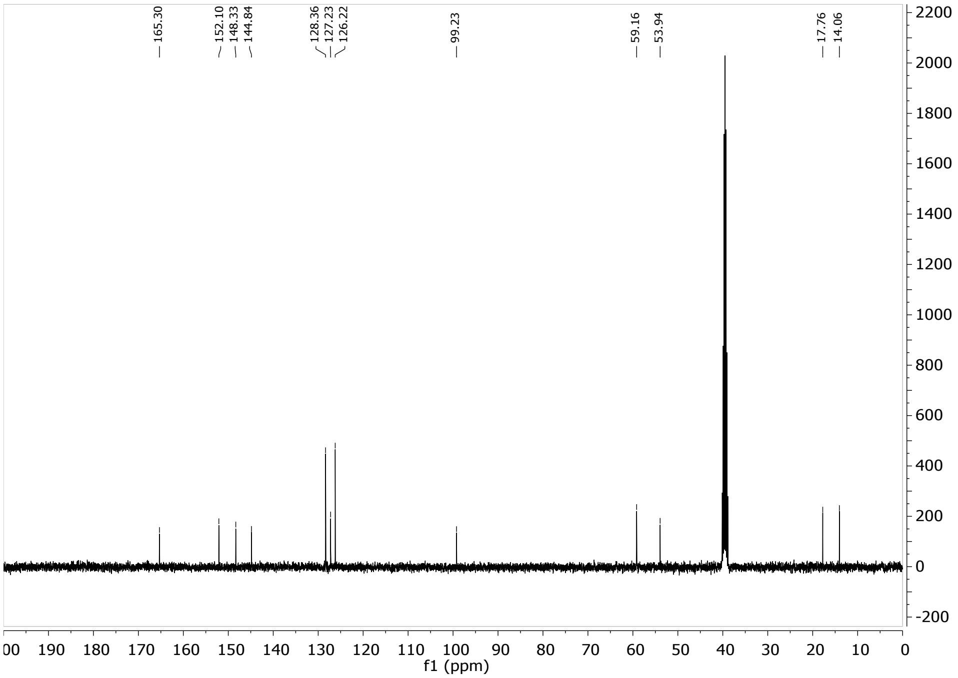
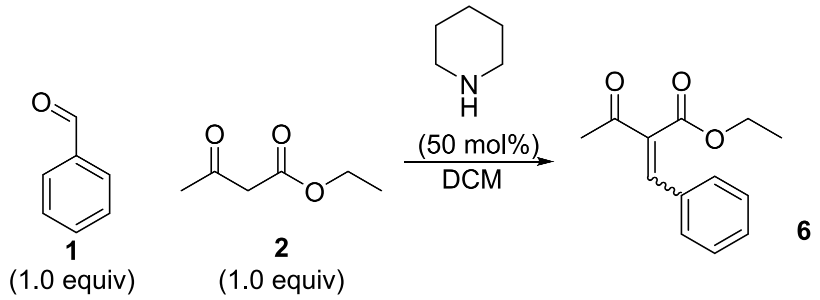


*Figure S3.125 MHz ^{13}C NMR spectrum of (**4**).*

Bulk-phase preparation of *cis/trans* isomers of intermediate **6**:



Compound **6** was prepared according to a literature procedure.[7] In a flame-dried round bottom flask, benzaldehyde (1.0 mL, 10.0 mmol), ethyl acetoacetate (1.3 mL, 10.0 mmol) and piperidine (0.5 mL, 5 mmol) were dissolved in dichloromethane (15 mL). The flask was capped, and the resulting mixture was stirred at rt overnight. The solvent was then evaporated and the residue purified by silica chromatography eluting with 0-20% (EtOAc:Hexanes). Both compounds were isolated as pale-yellow oils (1.84 g, 89% yield, 8:2 E/Z). ^{1}H NMR (Trans isomer): (400 MHz, Chloroform-*d*) δ 7.67 (s, 1H), 7.38 (d, *J* = 1.5 Hz, 5H), 4.30 (q, *J* = 7.1 Hz, 2H), 2.35 (s, 3H), 1.33 (t, *J* = 7.1 Hz, 3H).

Bulk-phase control reaction in the absence of catalyst:

In a 3-dram vial equipped with a magnetic stir bar, benzaldehyde (0.63 mL, 6.25 mmol), ethyl acetoacetate (1.2 mL, 9.5 mmol), and urea (381.1 mg, 6.25 mmol) were dissolved in aqueous methanol solution (2.5 mL). The mixture was stirred at room temperature for 48 hours. The reaction progress was monitored by GC/MS. Only trace amounts of (4) were observed at 48 hours (See Figure S4).

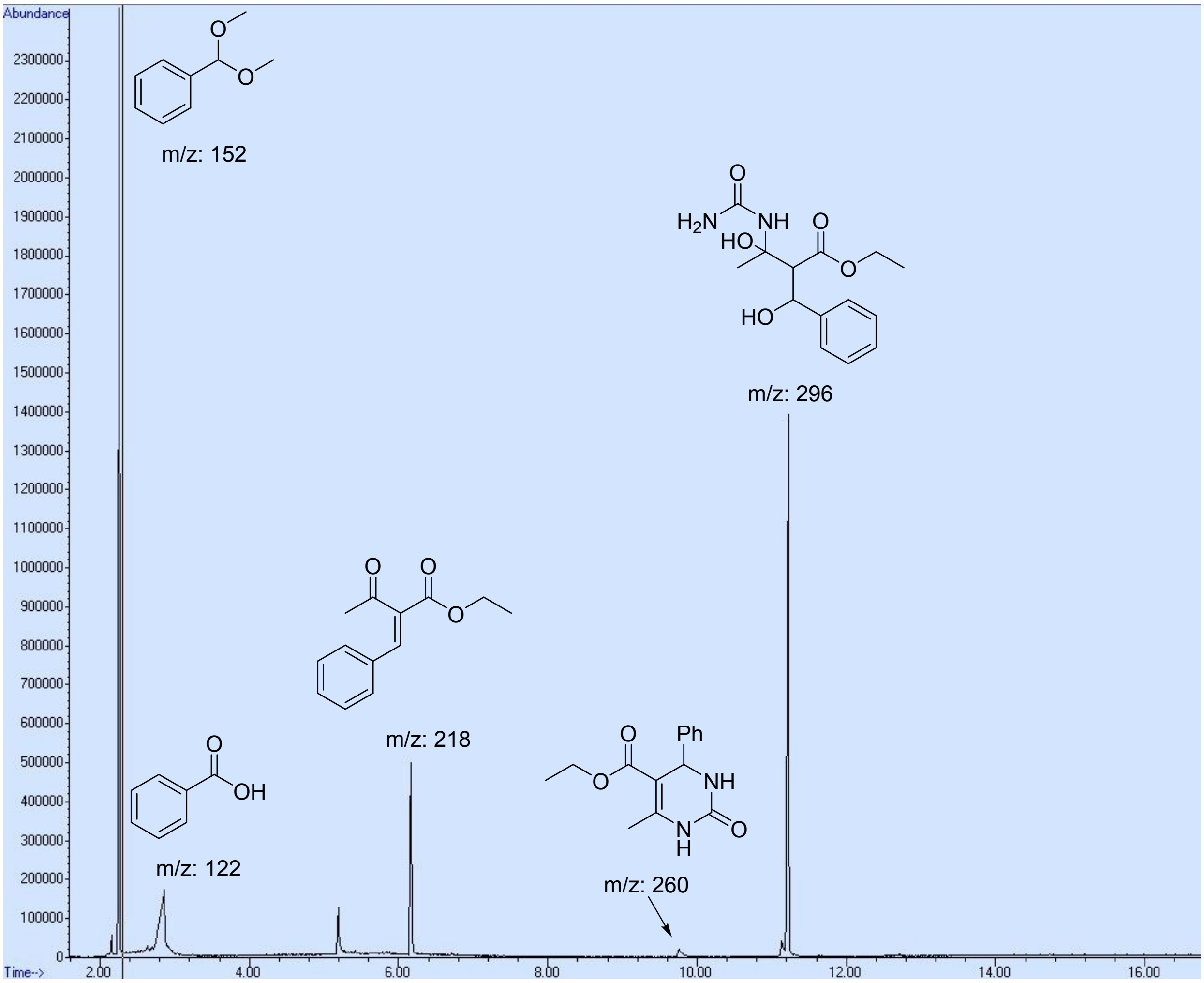


Figure S4: GC/MS spectrum of an uncatalyzed, bulk-phase biginelli reaction after 48 hours.

IMS confirmation of *trans*-6

To characterize the *cis/trans* peaks observed for **6**, both *trans*-**6** and *cis*-**6** were prepared in the bulk-phase. After purification, *cis*-**6** quickly isomerizes to give a mixture of the two isomers. The other isomer, *trans*-**6**, was stable enough to be handled and characterized using IMS (See Figure S5). This allowed for the characterization of the more extended feature in the IMS distribution in Figure 2.

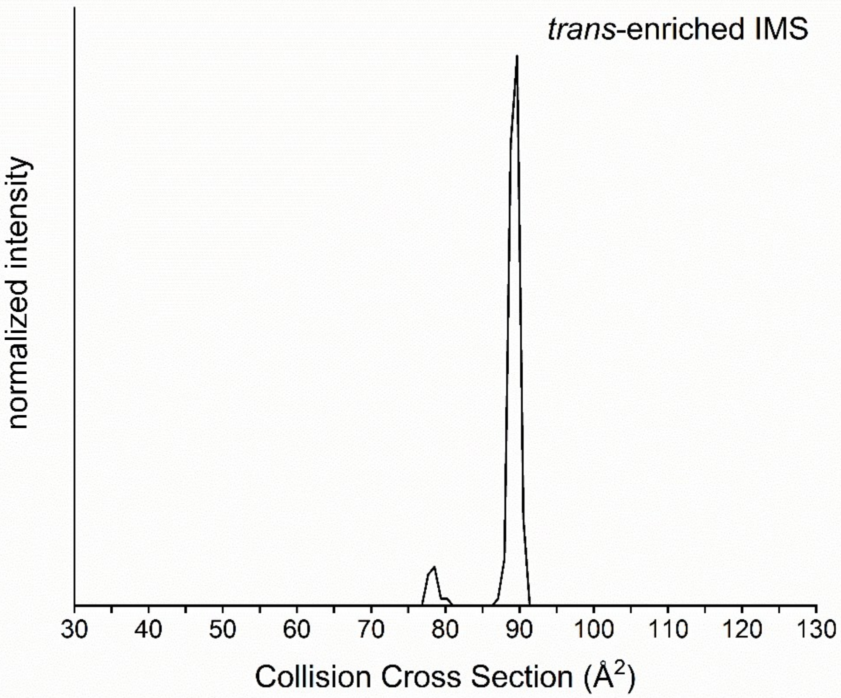


Figure S5. IMS of the trans-enriched isomer of ***6*** *prepared in bulk-phase and separated by column chromatography.*

Optimization of concentration, distance and applied voltage

Concentration Study:

Product formation was observed during electrospray ionization of reaction mixtures involving concentrations as low as 0.1 µM and 10 µM. Double barrel theta tips were investigated for the concentration studies.

Solvent Study:

A solution of 30 µM concentration was prepared from benzaldehyde, urea and ethyl acetoactetate in different solvents (methanol, ethanol, water and combinations of different proportions of water: alcohol) both with and without 0.002% acetic acid. Above solution was electrosprayed (4.5 kV at a flow rate of 25 µl min^{-1} through silica tip pulled to ~60 µm inner diameter. All these parameters were optimized for greater product abundance. Methanol and water mixed in 1:1 ratio gave the best results when compared to aqueous ethanol, water and different proportions of alcohols in water. The mass spectrometer inlet capillary temperature was maintained at 275 °C. The spray distance (the distance from silica emitter tip to the entrance of the heated capillary) was kept at ~ 6 -20 cm. All experiments for solvent studies were performed under identical conditions.

Gas-Phase control reaction:

Several controls were designed to ensure that molecule ion reactions were not forming products upon transition into the gas phase.

I. We prepared a solution of the reactant with the highest vapor pressure, benzaldehyde, at a 1 mM concentration in a solution of 1:1 MeOH/H_2O. Using the standard electrospray ionization source, we directed the auxiliary gas flow (6 arb) through a frit immersed into the benzaldehyde solution, which was held at 40 °C using a hot water bath. The frit allowed for an increased number and surface area of solvent bubbles to further saturate the auxiliary gas with benzaldehyde. Next, a solution of ethyl acetoacetate and urea (0.5 μM) was electrosprayed via the standard emitter with benzaldehyde-saturated nitrogen as auxiliary sheath gas. Using this setup, we observed no formation of the Biginelli product, but only observed ionization of the individual reaction precursors. We observed some ionization (presumably formed via the corona discharge) of the reagent benzaldehyde, further confirming the saturation of this reagent in the auxiliary sheath gas. A positive control experiment was also run where a solution of all three components was sprayed using the same benzaldehyde-saturated nitrogen sheath gas. This resulted in the base peak formation of the product. Based on these experiments this sufficiently tested concerns of ion-molecule reactions; isolating the source of product formation in microdroplets.

II. An additional control was completed using the ion trap instrument. Helium (40 psi) was split using two leak valves. The first leak valve controlled a helium gas flow through a solvent trap. The solvent trap contained benzaldehyde (1 mM, 50/50 methanol/water), which the helium was bubbled through. This benzaldehyde saturated helium was then recombined with high purity helium from the second leak valve. The second leak valve was used to control the level of high purity helium being mixed with the benzaldehyde saturated helium, thereby controlling the abundance of benzaldehyde reaching the ion trap. We then electrosprayed the remaining two reagents, ethyl acetoacetate and urea, using the standard ESI source. At all benzaldehyde partial pressures tested, we observed no Biginelli product formation.

Offline product collection:

To achieve dihropyrimidinone formation, reaction mixture of benzaldehyde, ethyl acetoacetate, and urea at 10mM concentration was prepared. The solution was loaded into 10.0 mL Hamilton gas-tight syringe fitted with luer-locks. Flow rates of 115 μL/min was used for maximum yield experiment. The optimized spray surface of a grounded glass wool in a column was used for collection. This experimental procedure allowed ~58% yield of the product.

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