

## Supporting Information

# Accelerated Bimolecular Reactions in Microdroplets Studied by Desorption Electrospray Ionization Mass Spectrometry

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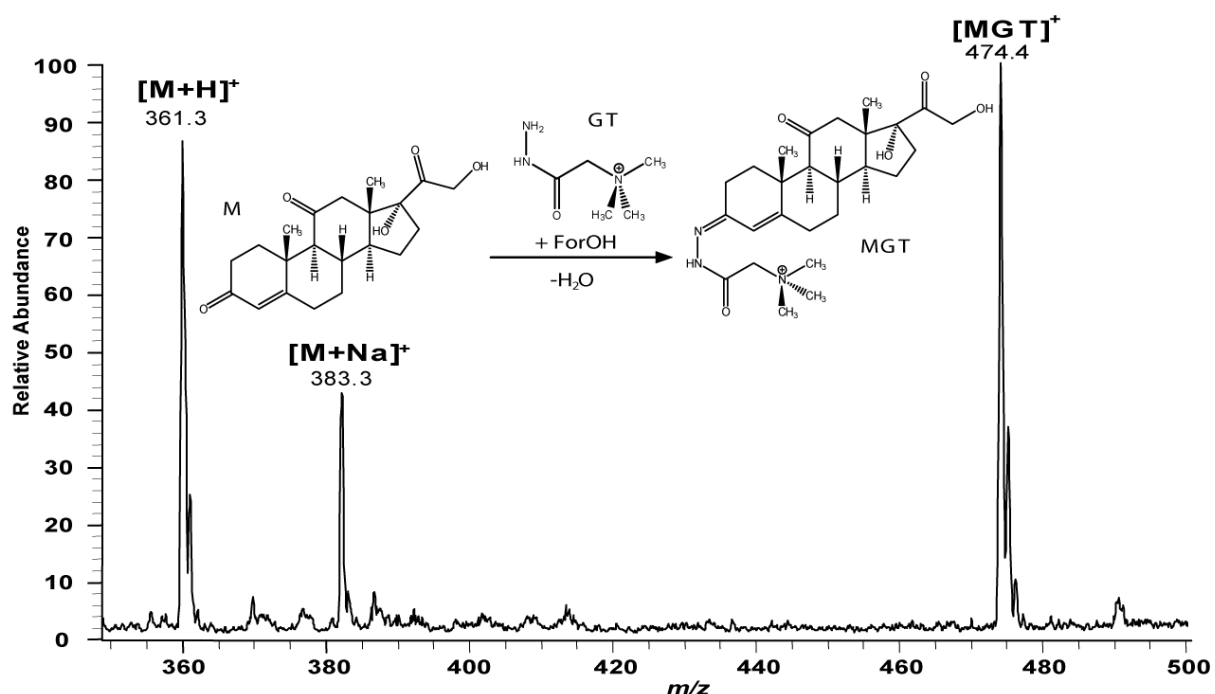
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### Summary

Reactive DESI spectrum of cortisone using a solvent doped with Girard T is shown (Fig. S1). The MS/MS product ion spectrum (Fig. 2b) of the Girard product of cortisone is rationalized as supporting the assigned structure. Data are then presented which establish a linear relationship between measured ion abundance ratios and concentration ratios (Fig. S2). Data showing the lack of effect on the reaction yield of delay time after exposure to the Girard T reagent (Fig. S3) and the influence of the DESI spray solvent and experimental conditions on the product yield (Fig. S4) are also shown. The results of the latter experiment are interpreted in terms of effects on droplet sizes which were independently inferred from spray cross section measurements made with water sensitive paper (Fig. S5). Typical data for reaction of androsterone with Girard T reagent is presented in Fig. S6.

## Reactive DESI spectrum of cortisone using a solvent doped with Girard T

Figure 1 shows typical data for the reactive DESI experiment averaged over the period of product formation. The data differs from that shown in Figure 1a only the fact that the spray remains on a single spot for the 30 second data acquisition period. The data are also very similar to those obtained for the normal incidence exposure of cortisone to Girard T reagent (Figure 2a) differing only in the absence of the peak at  $m/z$  393 which is assumed to be a contaminant in the earlier data.



**Figure S1.** Reactive DESI mass spectrum in the positive ion mode of cortisone examined as a solid spot on printed Teflon, using as solvent of MeOH/water 80:20 (v/v) with 0.5% of formic acid doped with Girard T at 50 ppm. Data averaged for 30 seconds.

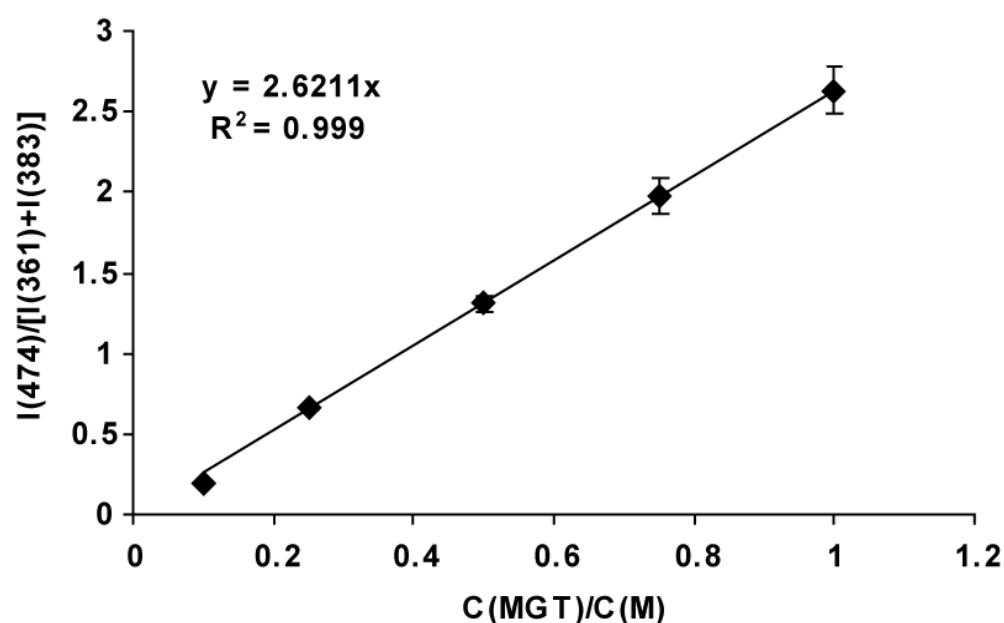
## Characterization of the Girard product by Tandem Mass Spectrometry

The product ion MS/MS spectrum of the cortisone/Girard T reaction product,  $m/z$  474 was recorded by reacting cortisone (solid) with Girard reagent in the normal incidence configuration, washing off the surface products and recording the nanoESI mass spectrum. The data (Fig. 2b)

was obtained by isolating  $m/z$  474 using a 1.5 Thomson window and dissociation by collisional activation. The fragment ion detected at  $m/z$  456 arises from the loss of a water molecule, while the elimination of formaldehyde ( $\text{CH}_2\text{O}$ , 30 Da) gives rise to the ion detected at  $m/z$  444. The main fragment ion,  $m/z$  415, is assigned to the loss of trimethylamine from the precursor ion. Elimination of a CO molecule then gives rise to the fragment ion detected at  $m/z$  387.

### Linear relationship between measured ion abundance ratios and concentration ratios

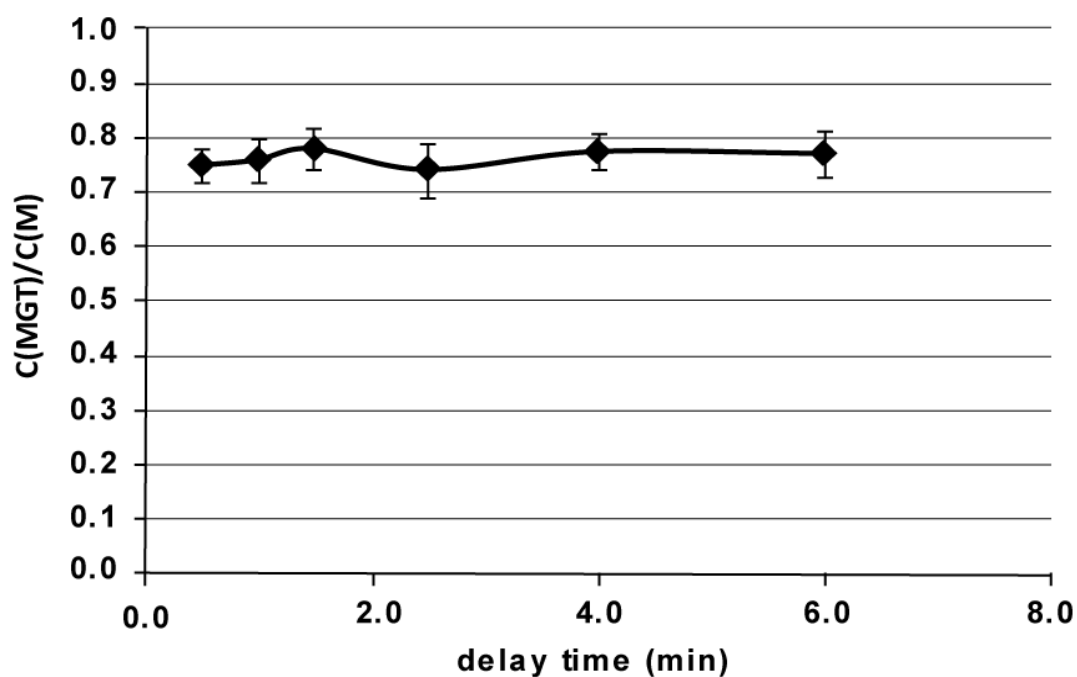
This relationship was established using standard solutions and the data are shown in Fig. S2



**Figure S2.** Relationship between the ratios of product to reactant intensities in nanoESI analysis and the known ratios of concentrations of cortisone (M) and the reaction product (MGT) in MeOH/water 80:20 (v/v) with 0.5% of formic acid. Bars represent the standard deviation for three replicate analyses.

### Effect of delay time after exposure to Girard reagent on reaction yield

The possibility that reaction might occur in solution was tested and eliminated by exposing a cortisone spot to Girard T reagent and then waiting before analysis of the spot by nanoESI. The data (Fig. S3) show that there is no reaction after the exposure period.

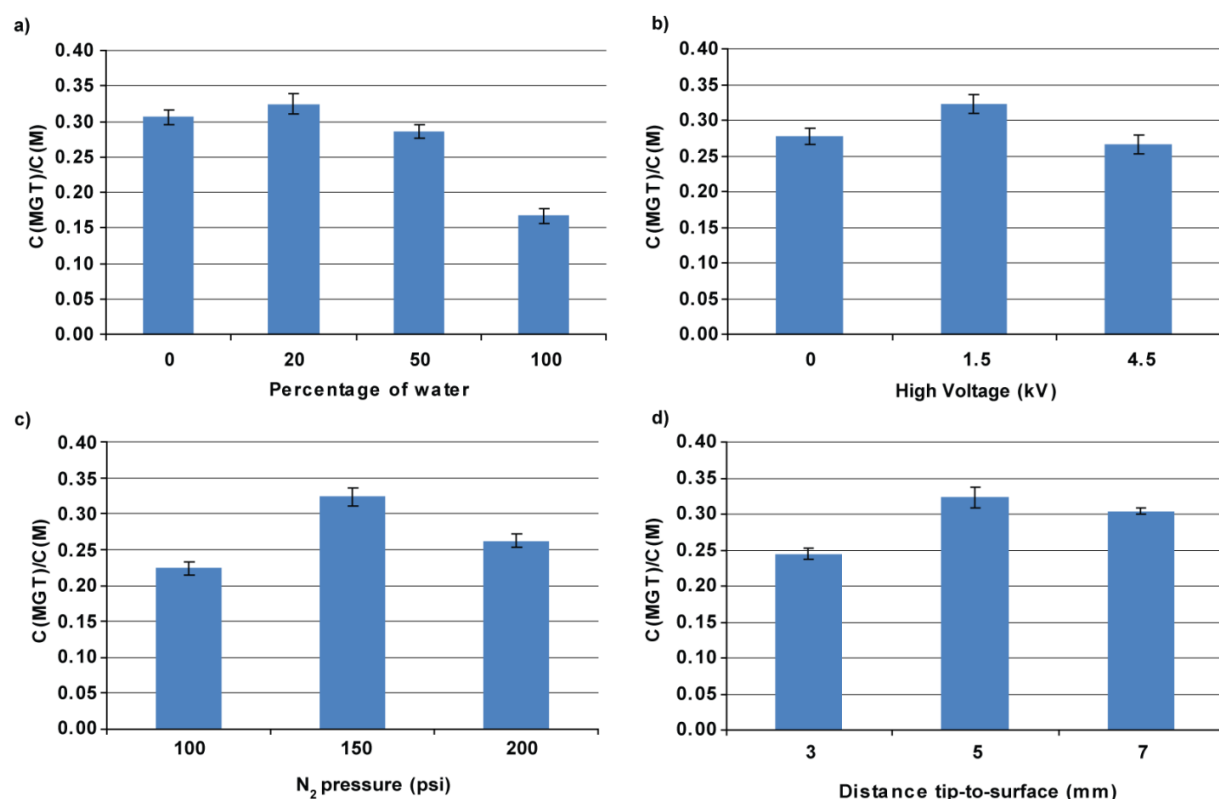


**Figure S3.** Lack of effect of the delay time between exposure to microdroplets and analysis of the cortisone substrate on the reaction yield. Exposure conditions: GT at 50 ppm in MeOH/water 80:20 (v/v) with 0.5% of formic acid, incident angle to the horizontal: 90°, flow rate: 5  $\mu$ L/min, exposition time: 30 s, tip-to-surface distance: 5 mm, nebulizing gas pressure: 150 psi, high voltage: +1.5 kV. Ratios of concentrations are calculated from the nanoESI spectra. Bars represent the standard deviation of analysis for three replicates.

### **Influence of the DESI spray and experimental conditions on product yield**

Experiments have been performed by varying the composition of the DESI spray solvents (Figure S4a). Increasing the water content decreases product formation by limiting rate of evaporation and hence keeping the droplet pH high. The product/reactant ratios have also been measured in experiments where the analyte ketone was exposed to reagent droplets at 0, +1.5 and +4.5 kV (Figure S4b), with a maximum occurring at +1.5 kV. Increasing the voltage applied to the sprayer produces droplets with greater charge due to the electrospray process and as a result, the measured pH is lower and the reactant concentrations are higher. However, it seems that when the voltage is too high the primary droplets might be too small to effectively participate in the splash process, yielding smaller secondary droplets with so little formic acid that this source of protons limits product relative to reactant.

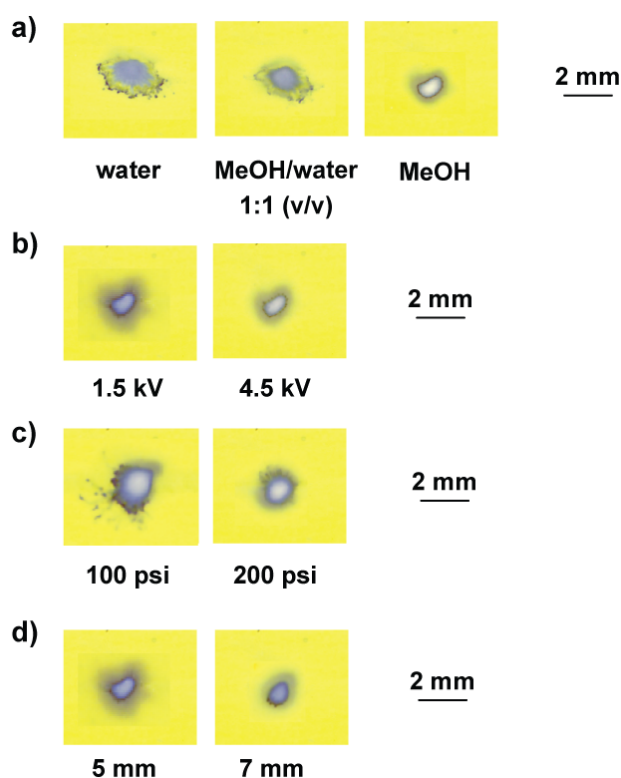
Similar phenomena are observed with the nebulizing gas pressure (Figure S4c). A previous study has shown that with an increase in the supply pressure the droplet diameters decreased while the velocity of the droplets increased up to a maximum value. Large droplets are produced at low pressure (100 psi) due to ineffective nebulization (Figure S4c), which would account for a lower reaction yield. The decrease of the reaction yield at 200 psi can be tentatively ascribed to the inefficient splash process due to primary droplets that are too small. As the velocity of the microdroplets decreases with distance from the spray tip, the evaporation time of MeOH/water 80:20 (v/v) droplets increases before impacting the surface. As a result smaller microdroplets are produced and subsequently an increase in the reaction yield is observed with the distance tip-to-surface (Figure S4d).



**Figure S4.** Influence of the spray parameters on the reaction yield for the cortisone analyte using a 5  $\mu\text{L}/\text{min}$  flow rate for 30 sec with an incident angle to the horizontal of  $90^\circ$ . a) Varying percentages of water in the spray solvent. Exposure conditions: GT at 50 ppm in MeOH/water with 0.5% of formic acid, nebulizing gas pressure: 150 psi, tip-to-surface distance: 5 mm, high voltage: +1.5 kV. Ratios of concentrations are calculated from the DESI spectra. b) Varying applied high voltages. Exposure conditions: GT at 50 ppm in MeOH/water 80:20 (v/v) with 0.5% of formic acid, tip-to-surface distance: 5 mm, nebulizing gas pressure: 150 psi. c) Varying nebulizing gas pressures. Exposure conditions: GT at 50 ppm in MeOH/water 80:20 (v/v) with 0.5% of formic acid, tip-to-surface distance: 5 mm, high voltage: +1.5 kV. d) Varying tip-to-surface distances. Exposure conditions: GT at 50 ppm in MeOH/water 80:20 (v/v) with 0.5% of formic acid, nebulizing gas pressure: 150 psi, high voltage: +1.5 kV. Bars represent the standard deviation of analysis for three replicates.

### Droplet sizes measured with water sensitive paper

The results just described can be interpreted in terms of droplet size effects which can be deduced by measurements of cross sections of the DESI spray as observed on water sensitive paper (Figure S5). This methodology and assumptions has been described previously.<sup>1</sup> The data are consistent with reduced droplet sizes with increased methanol content of the spray solvent, increased applied potential, increased pressure and increased capillary to inlet spacing.



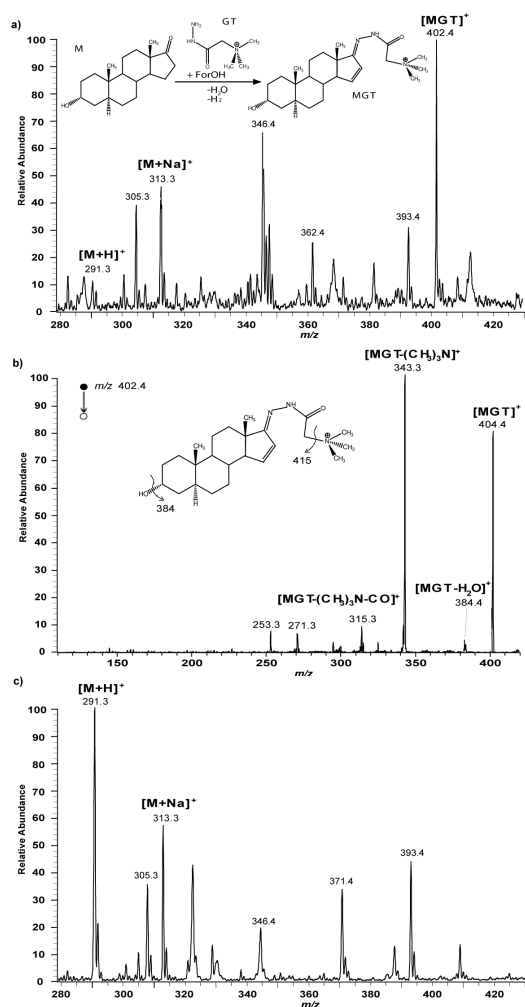
**Figure S5.** Shape and size of the DESI spray of GT for various experimental conditions using 5  $\mu\text{L}/\text{min}$  flow rate sprayed for 30 sec with an incident angle to the horizontal of  $90^\circ$  on water sensitive paper. a) Exposure conditions: GT at 50 ppm in MeOH (left), MeOH/water 50:50 (v/v) (middle) and water (right) with 0.5% of formic acid, nebulizing gas pressure: 150 psi, high voltage: +1.5 kV, tip-to-surface distance: 5 mm; spray diameters: 1.66 mm (left), 1.98 mm

(middle) and 2.45 mm (right). b) Exposure conditions: GT at 50 ppm in MeOH/water 80:20 (v/v) with 0.5% of formic acid, tip-to-surface distance: 5 mm, nebulizing gas pressure: 150 psi, high voltage: +1.5 kV (left) and +4.5 kV (right); spray diameters: 1.83 mm for +1.5 kV and 1.57 mm for +4.5kV. c) Exposure conditions: GT at 50 ppm in MeOH/water 80:20 (v/v) with 0.5% of formic acid, tip-to-surface distance: 5 mm, high voltage: +1.5 kV, nebulizing gas pressure: 100 psi (left) and 200 psi (right); spray diameters: 1.89 mm for 100 psi and 1.62 mm for 200 psi. d) Exposure conditions: GT at 50 ppm in MeOH/water 80:20 (v/v) with 0.5% of formic acid, nebulizing gas pressure: 150 psi, high voltage: +1.5 kV, tip-to-surface distance: 5 mm (left) and 7 mm (right); spray diameters: 1.83 mm (left) and 1.52 mm (right).

### **Reaction of androsterone with Girard T reagent**

This experiment paralleled that of cortisone and the results are presented (Figure S6) in similar format with the nanoESI spectrum of the exposed steroid, the product ion MS/MS spectrum of the protonated reaction product and a spectrum of the bulk reagent mixture being shown.





**Figure S6.** a) Positive ion nanoESI mass spectrum recorded after exposure of androsterone (100 ppm on glass slide) to microdroplets containing the GT reagent. Exposure conditions: GT at 50 ppm in MeOH/water 80:20 (v/v) with 0.5% of formic acid, incident angle to the horizontal: 90°, flow rate: 5  $\mu$ L/min, exposition time: 5 min across the spot, tip-to-surface distance: 5 mm, nebulizing gas pressure: 150 psi, high voltage: +1.5 kV. Inset shows the chemical reaction. b) nanoESI-MS/MS spectrum of [MGT]<sup>+</sup> product detected at  $m/z$  404 after exposure of androsterone to microdroplets containing the GT reagent under similar experimental conditions. c) Positive ion nanoESI mass spectrum of the bulk solution. Bulk conditions: 1.5  $\mu$ L of androsterone at 100 ppm in MeOH mixed with 25  $\mu$ L of GT at 50 ppm in MeOH/water 80:20 (v/v) with 0.5% of formic acid, reaction time: 5 min, temperature: 25°C.

### **Cinnamic acid reaction mechanism**

Cinnamic acid did not undergo a normal Michael addition but yielded a dehydro product (Figure 7). To examine the mechanism of this reaction dideuteriocinnamic acid was prepared by exchange with D<sub>2</sub>O (Figure 7) and reacted with dimethylmalonate. The MS/MS product ion spectra of the d<sub>0</sub>- and d<sub>2</sub>-products are compared in Fig. S7. The data demonstrate that the label is lost in the course of product formation.

### **REFERENCES**

1. A. Badu-Tawiah, *Journal of the American Society of Mass Spectrometry*, 2010, **21**, 1423-1431.