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Accelerated *tert*-butyloxycarbonyl deprotection of amines in microdroplets produced by a pneumatic spray

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ABSTRACT

Protection and deprotection of organic compounds in multistep reactions using functional groups such as *tert*-butoxycarbonyl (Boc), is widely performed in synthetic organic chemistry. Reaction rate acceleration studies in spray-based ionization methods (electrosonic spray, paper spray, nanospray) have become increasingly common. Here, we demonstrate reaction rate acceleration of Boc deprotection using easy ambient sonic-spray ionization (EASI), a pneumatic technique which does not involve an applied voltage, in a teaching laboratory setting. The goal of this laboratory exercise was to explore acceleration in a previously unexplored spray-based reaction, while emphasizing in a pedagogic setting the importance of protecting groups for multistep synthesis. Rate acceleration factors of more than an order of magnitude were observed in the uncharged micron-sized droplets generated by EASI. The effect of reaction conditions on reaction acceleration was examined including changes in the type of acid, reagent concentration ratios and syringe pump flow rates. Student knowledge was assessed by pre-laboratory assignments, post-laboratory reports and oral interviews.

1. Introduction

Multistep organic synthesis involves a sequence of reactions from starting materials to product, frequently with use of column chromatography or other means to purify intermediates [1]. There are many possible reaction routes to any one desired product or intermediate, and cost, time, and yield are criteria in choosing the best synthetic route [2]. Throughout a multistep synthesis, functional groups may need to be conserved. A protecting group is often introduced prior to a particular reaction step and later removed, in order to preserve a specific group that otherwise would not survive [3]. To undergraduate chemistry students, knowledge of the use of protection and deprotection reactions is pivotal to the understanding of multistep synthesis [4–6].

Protecting and deprotecting a functional group adds two additional steps to a multistep synthetic scheme. There are a battery of protecting groups, each with requirements that need to be met for their removal once the molecule has progressed to a stage in the reaction scheme where the functional group can be safely maintained through the subsequent steps to the final product [7]. The protecting group used in this experiment is the *tert*-butyloxycarbonyl functional group, commonly referred to as "Boc."[8] It is primarily utilized to protect amines in multistep syntheses and is extensively used in peptide synthesis and medicinal chemistry [9]. The Boc group can be removed by relatively strong acids, such as hydrochloric acid or trifluoroacetic acid, or by the combination of heat and milder acids [8].

Time is a major consideration in multistep reactions, so chemists typically increase the rate of reaction by using elevated temperatures. Refluxing is usually used to achieve this increase in rate without losing sample or solvent [10]. While thermal acceleration is widely used, another common method of acceleration is through catalysis [11]. Catalysts accelerate reactions by providing an alternative mechanistic pathway with a lower activation energy. More recently, there has been a growing recognition that reactions can be accelerated by other means, specifically by performing reactions at interfaces [12–14]. Modest acceleration has been reported for some reaction mixtures in microfluidics [15,16], while electrospray ionization [17–20], and other spray methods produce droplets which sometimes yield very large acceleration factors. In cases of reactions in small confined volumes (i.e. thin films or microdroplets) it is thought that partial solvation of the reagents at the air-solvent

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interface is the cause of reaction rate acceleration [21]. We explore the use of a spray-based method to accelerate the deprotection of an amine in this laboratory exercise. Specifically, the deprotection of Boc-Ala-OH (1) by acid to produce free Ala-OH (2). The deprotection occurs by initial protonation of the *tert*-butyl carbamate and subsequent loss of the cationic butyl group as the carboxylic acid with production of the free amine.

Reaction rate acceleration has been demonstrated using online mass spectrometric analysis of reaction mixtures ionized by electrospray ionization and other spray-based ionization methods [2,18–20,22–25]. This phenomenon has been highlighted in recent reviews [21,26,27]. Experiments can be performed using electrospray ionization to spray and collect appreciable amounts of material in minutes [17]. Using a continuous thin film variant of droplet chemistry, Wei et al. collected nearly 100 mg/hr of reaction product with a steady state rate acceleration factor of 100 [28]. We use a variety of electrospray and reaction conditions to explore both the kinetics of the reaction and the processes of electrospray reaction rate acceleration. Various factors influence reaction rate acceleration in electrospray including: solution flow rate, gas flow rate, collection surface and reagent concentration [21]. Factors such as solvent evaporation will increase reaction rates but may not change the rate constant. On the other hand, increasing the surface/volume ratio may increase rate constants if surface reactivity differs from bulk reactivity. Reaction rate acceleration can be calculated by comparing the rate for the bulk material to that recorded using the accelerated method. This is approximated by simply taking the ratio of product to starting material ratio for the sprayed material divided by the ratio for the bulk after the same reaction time. This calculated rate acceleration factor is only approximate as it assumes equal ionization efficiencies for the reagent and product as well as assuming the same form of reaction kinetics (Equation 1) [28,29].

$$\textit{Reaction Rate Acceleration} = \frac{\left(\dfrac{\textit{Intensity of Product}}{\textit{Intensity of Reactant}}\right)_{Spray}}{\left(\dfrac{\textit{Intensity of Product}}{\textit{Intensity of Reactant}}\right)_{Bulk}}$$

Equation. 1 Reaction acceleration is determined by the ratio of ratios of product to reactant of spray and bulk.

The first learning objective of this laboratory exercise was for students to gain a better understanding of how spray-based reactions can be accelerated when compared to their solution-phase counterparts. Unlike previously accelerated reaction exercises performed, developed and implemented by our research group [21,30,31], this chemical system uses no voltage during the acceleration, a modification that is more amenable to a teaching laboratory environment, while maintaining the mission of bringing cutting-edge research to the teaching laboratory [32]. This “no-voltage” spray-based method, also known as easy ambient sonic-spray ionization (EASI), has been the topic of a recent review [33]. The chemical system of *tert*-butyloxycarbonyl (Boc) deprotection has been selected for its common and important use in medicinal chemistry [9,34] and for the relatively low reagent cost. One learning objective centered on students considering how experimental parameters influence the acceleration of the formation of the deprotected product. Some of these experimental parameters – variation in the flow rate, concentration of the Boc-protected compound relative to the reactant acid, and the choice of acid itself – changed the measured rate acceleration factor. Note that the exercise does not measure intrinsic rate constants. The second learning objective was for students to understand the purpose and importance of protecting groups in multistep syntheses. By conducting part of a multistep synthesis in an accelerated fashion, students learned how and why protecting groups have such an important role in organic synthesis and how time-saving steps could benefit synthesis.

2. Experimental

2.1. Chemicals and EASI setup

All chemicals (Boc-Ala-OH, Boc-Ala-OMe, hydrochloric acid (HCl), and trifluoracetic acid (TFA)) were purchased from Sigma-Aldrich (St. Louis, MO) except for methanol (MeOH) which was purchased from Fisher Scientific (Pittsburgh, PA). EASI spray emitters were constructed with fused silica lines with 100-μm I.D. and 360-μm O.D. (PolyMicro, Phoenix, AZ), one tee assembly, one union assembly, two NanoTight sleeves, and a stainless-steel capillary (IDEX Health and Science, Oak Harbor, WA). To control the flow of reagent solution, infuse syringe pumps (Standard Infusion PHD 22/2000, Harvard Apparatus, Holliston, MA) were utilized with gastight chemseal syringes (Hamilton Robotics, Reno, NV). Nitrogen (Indiana Oxygen, Lafayette, IN) was used as the nebulizing gas. The construction and part numbers can be found in Fig. 1. The reaction mixtures were sprayed into 15 mL Falcon conical centrifuge tubes (Fisher Scientific, Pittsburgh, PA) from which the bottom had been removed, so as to avoid pressure build-up, with glass wool in its place to collect the product.

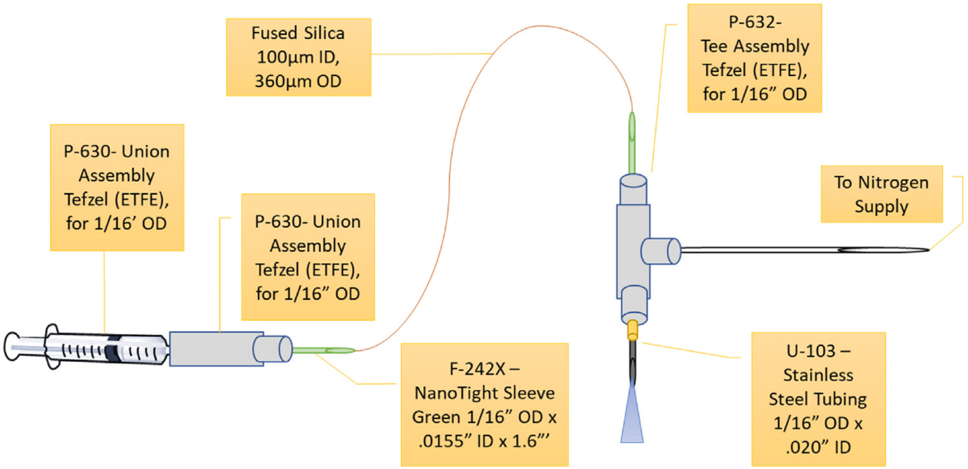


Fig. 1. Easy ambient sonic-spray ionization (EASI) droplet generation system consisting of a gastight syringe, fused silica lines, Teflon unions, nanotight sleeves and a stainless-steel capillary.

Table 1
Reactions that students performed using six sets of conditions, altering flow rate, acid type, and acid to Boc-Ala-OH ratio.

Student's Reaction Conditions		
Flow Rate	HCl	TFA
5 µl/min	10:1 HCl to Boc-Ala-OH	10:1 TFA to Boc-Ala-OH
5 µl/min	1:1 HCl to Boc-Ala-OH	1:1 TFA to Boc-Ala-OH
20 µl/min	10:1 HCl to Boc-Ala-OH	10:1 TFA to Boc-Ala-OH

2.2. Experimental parameters

This three-hour laboratory exercise was performed by 15 undergraduate students enrolled in an organic chemistry II honors section. The students were divided into three groups, and each group performed the same set of six reactions using a range of conditions, with varying flow rates, acids and ratios of acid to Boc-Ala-OH (Table 1). No voltage was applied to the EASI spray emitter.

2.3. Mass spectrometry

All spectra were recorded in positive ion mode using a LTQ XL ion trap mass spectrometer (Thermo Instruments, San Jose, CA). All mass spectra recorded for product analysis utilized nanospray ionization as the ionization source at 2.0 kV and set 3 mm from the inlet to the vacuum system. The nanospray emitters were constructed from borosilicate glass capillaries (1.5 mm o.d., 0.86 mm i.d., Sutter Instrument Co.) that were pulled to a tip using a Flaming/Brown micropipette puller (Sutter Instrument Co. model P-97, Novato, CA, U.S.A.).

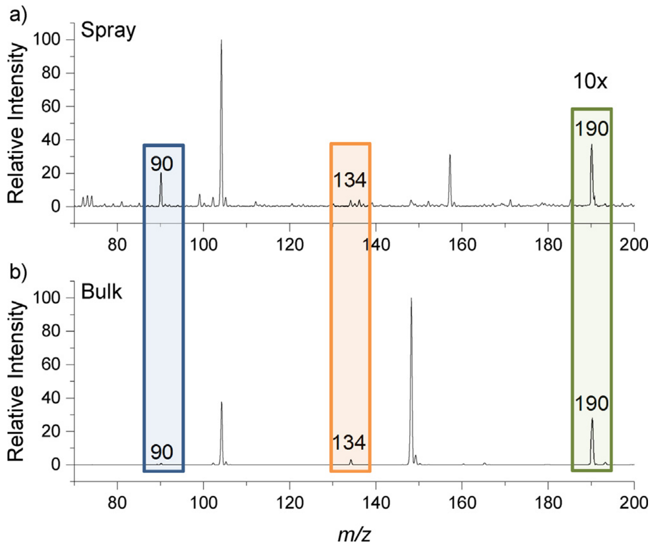


Fig. 2. Full scan positive ion mode mass spectra. The blue box indicates the Ala-OH which has had the Boc group removed, whereas the green indicates the reactant Boc-Ala-OH. The reactant peak has been scaled by a factor of ten to aid in visualization. The orange box indicates the intermediate, which the students did not use in their calculations. (a) Spray reaction utilizing HCl in a 10:1 ratio of acid to Boc-Ala-OH at a flow rate of 5 µL/minute. (b) Corresponding bulk reaction mixture. Ion signals at *m/z* 104 and 148 are the product and intermediate of a side reaction (esterification of the carboxylic acid of the Boc-Ala-OH) and for the purpose of the teaching laboratory exercise were not brought to the attention of the student. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Increase in ratio of the acid to the Boc-Ala-OH causes the product to form at an accelerated rate. Similarly, both acids had the highest acceleration rate with an EASI flow rate of 5 µL/min. TFA in a 10:1 ratio to Boc-Ala-OH at a flow rate of 5 µL/min had the largest acceleration factor overall.

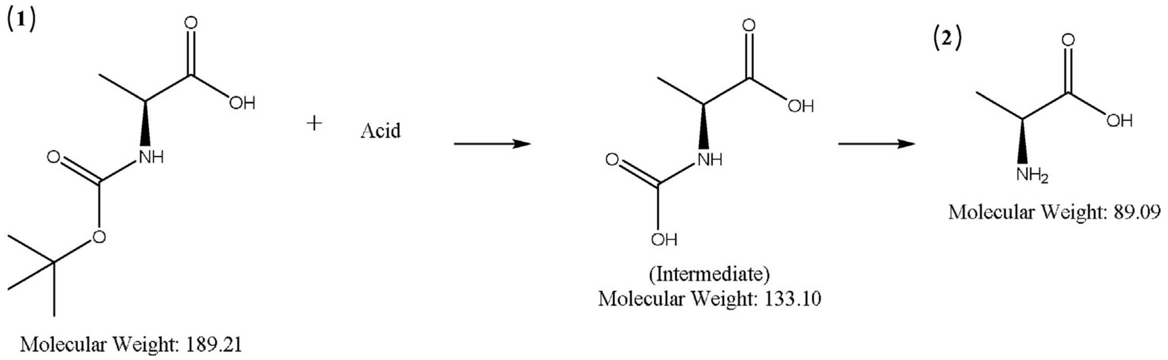
Student Reaction Acceleration Factors for Boc-Ala-OH for Each Spray Condition			
Acid	Mole Ratio of Acid to Boc-Ala-OH	Flow Rate	Acceleration Factor
HCl	1:1	5 µL/min	1.2
	10:1		18
		20 µL/min	12
TFA	1:1	5 µL/min	2.0
	10:1		35
		20 µL/min	4.2

3. Results and discussion

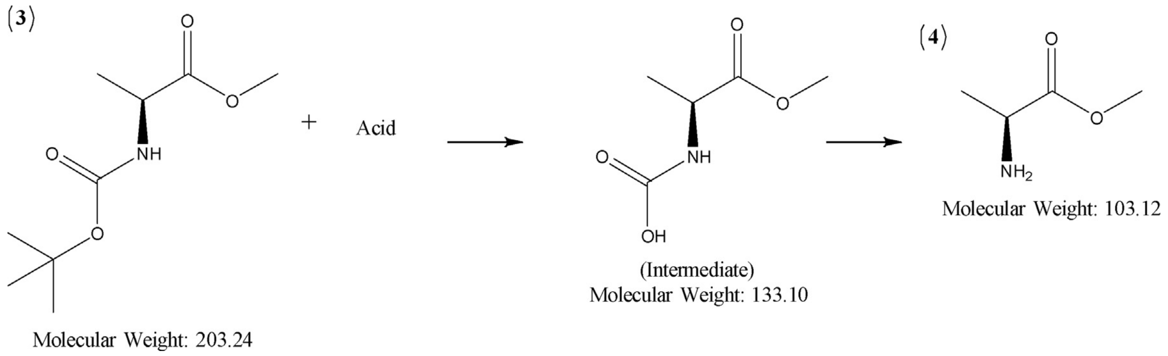
3.1. Accelerated reactions

To determine the acceleration factor for spray conditions relative to bulk conditions, students performed reactions under each of the six conditions listed in Table 1 in both bulk and spray. Upon completion of the spray reactions, the product was extracted from the glass wool by running 2 mL of MeOH through the tube. Reactions that were conducted under bulk conditions were all allowed to react for a time equal to the entire spray and sample workup. Both the spray based and bulk reactions were analyzed by nanospray ionization mass spectrometry. Fig. 2 shows student-collected spectra of both the spray and the bulk conditions using HCl in a 10:1 ratio acid to Boc-Ala-OH, with a flow rate of 5 µL/min condition. The reactant was observed at *m/z* 190, corresponding to protonated Boc-Ala-OH, and the product was observed at *m/z* 90, corresponding to protonated Ala-OH with removal of the Boc protecting group. The carbamic acid intermediate in the Boc deprotection process was also observed at *m/z* 134, which corresponds to the loss of the *tert*-butyl group and formation of protonated carbamic acid (Scheme 1).

Students collected spectra for each condition and calculated the acceleration factors (uncorrected for ionization efficiencies) of each



Scheme 1. Deprotection of Boc-Ala-OH (**1**) using strong acid to form the amino acid (**2**) via the intermediate carbamic acid.



Scheme 2. Deprotection of Boc-Ala-OMe (**3**) using strong acid to form the amino acid ester (**4**) via the intermediate carbamic ester.

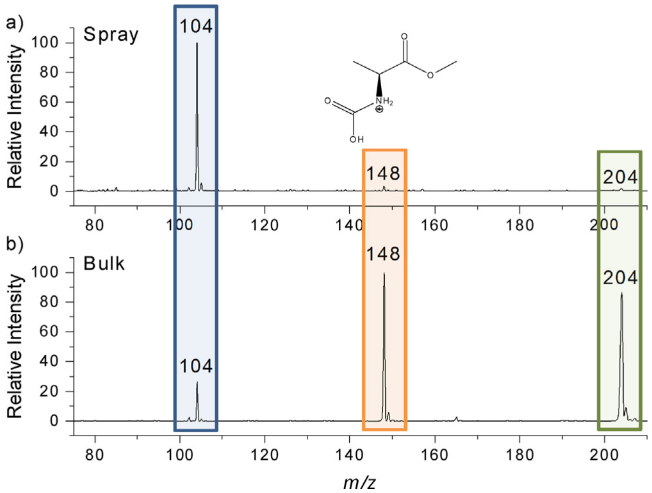


Fig. 3. Full scan positive mode spectra. The blue box indicates the Ala-OMe from which the Boc group is removed, the green indicates the reactant Boc-Ala-OMe. The orange box indicates the intermediate. (a) The spray reaction utilizing HCl in a 10:1 ratio of acid to Boc-Ala-OMe at a flow rate of 5 μL/minute. (b) Mass spectrum for corresponding bulk reaction mixture. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Increase in ratio of the acid to the Boc-Ala-OMe causes the product to form at an accelerated rate. Similarly, the highest acceleration rate with an EASI flow rate of 5 μL/min. The larger acceleration factors may provide the justification of spending more on the reagents to more clearly demonstrate the fundamentals of reaction acceleration to students.

Reaction Acceleration Factors for Boc-Ala-OMe for Each Spray Condition			
Acid	Mole Ratio of Acid to Boc-Ala-OMe	Flow Rate	Acceleration Factor
HCl	1:1	5 μL/min	59
	10:1		216
		20 μL/min	183

condition (Table 2). They then compared the acceleration factors for each condition to determine which provided the largest acceleration factor compared to its bulk counterpart. Every condition that the students utilized demonstrated spray-based acceleration. The most significant acceleration was observed from the TFA acid condition in a 10:1 acid to Boc-Ala-OH ratio, with a flow rate of 5 μL/min during spray reaction. While not explored by the students in this laboratory exercise, the deprotection of Boc-Ala-OH led to a side reaction where the carboxylic acid on the alanine was converted into the methyl ester (**3**). This reaction was accelerated to form the deprotected Ala-OMe (**4**) as seen in Scheme 2.

To determine the acceleration of deprotection of the Boc-Ala-OMe without the convoluting effect of the methylation of the carboxylic acid *in-situ*, the same experiment was performed with using Boc-Ala-OMe as the starting material. Fig. 3 shows the spray and bulk spectra for bulk conditions for the HCl acid condition in a 10:1 ratio acid to Boc-Ala-OMe, with a flow rate of 5 μL/min condition.

There is a larger acceleration factor as seen in Table 3 likely due to the removal of the intermediate step of the methyl-ester formation. The trend in acceleration factor for Boc-Ala-OMe is the same as Boc-Ala-OH. Boc-Ala-OMe was not used initially in the teaching laboratory exercise because it costs three times as much as Boc-Ala-OH and the fundamentals of spray-based reaction acceleration were still demonstrated. However, if budget allows, the authors suggest utilizing the Boc-Ala-OMe as the reactant as it yields a larger acceleration rate for the students to observe and may increase their understanding of the concept. Similarly, depending on the time allotted for the laboratory exercise comparing both the Boc-Ala-OH and the Boc-Ala-OMe could be a worthwhile learning exercise to discuss side reactions and by-products.

3.2. Laboratory evaluation and assessment

The students were required to answer pre-laboratory questions based on the background information provided on spray-based reaction acceleration and the importance of Boc protection and deprotection. A week after the completion of the laboratory exercise, students turned in post-laboratory questions and data manipulations to assess their comprehension of the experiment. Both the pre-laboratory and post-laboratory questions are available in the Supporting Information. In an ungraded exercise a week after the completion of the laboratory exercise, the students were interviewed orally to assess their understanding of mass spectrometry, reaction rate acceleration, and the experiment in which they participated. Students were interviewed in three groups of 5 to maximize participation and promote discussion that may not have been elicited through individual written or verbal prompting [35]. Table 4 summarizes the responses to the five questions that the students were asked to answer.

As seen in Table 4, all the students thought this was a worthwhile experiment, sharing excitement about the novelty of this experiment since this reaction had not previously been reported in spray. For example, one student stated that this was an "...alternative method that may be more commonly used in the future... We are ahead of the game." In their post-laboratory reports, the students most commonly suggested multiplexing the spray emitters to increase product formation. The students showed a fundamental understanding on how reactions are accelerated by spray-based methods and how to calculate acceleration factors based on their pre-laboratory, post-laboratory, and verbal interviews.

Table 4
Student responses to post-laboratory verbal interviews.

Comparison of Student Post-Laboratory Verbal Interview Questions and Responses			
Questions/Statements for Response[a]		Groups, N[b]	Response Characterization – Students in the Group:
1	This is probably the first time that EASI has been used to spray and collect accelerated reaction products in an undergraduate teaching laboratory. Could you please explain why you believe this was or was not a valuable laboratory experiment? How could this experiment be improved?	3	Thought the experiment was a valuable laboratory exercise.
		3	Stated that data processing was difficult. (Although stated to be difficult all the students correctly determined their acceleration factors.)
		1	Suggested that only one variable should be assigned to a group and then the data pooled.
2	Please describe the collection set-up, EASI parameters, as well as syringe pump parameters.	3	Described the overall experimental setup.
		3	Explained why the bottom of the collection tube was removed to allow gas flow and the use of glass wool as a collection mechanism.
		3	Described the effect of flow rate on the syringe pump had on the rate of acceleration and droplet size.
3	Why are reactions able to be accelerated by spray-based methods? What factors contribute to this?	3	Described surface effects of the partially desolvated droplets.
		2	Described the change in activation energy in partially desolvated droplets.
4	Please explain how you calculate acceleration factors?	3	Stated that it was a ratio of the bulk and spray based methods' ratio of intensities of the product and reactants.
5	Why does the nanospray analysis not effect the acceleration?	3	Described how both the bulk and sprayed products are analyzed using nanospray so the effect is negated.

[a] Graduate TAs verbally interviewed students.
[b] The 15 total students were assigned to 3 groups of 5 each.

4. Conclusions

This laboratory exercise provided organic II students with the opportunity to learn about the importance of protecting groups and multistep synthesis within the framework of spray-based accelerated reactions. As seen in the laboratory reports and the verbal interviews, students developed a grasp of the fundamental theories explaining why reactions accelerate in spray-based methods, their importance to time and ease of synthesis, all while exploring new chemistry. The benefit of no voltage being applied during the spray process, as well as the low cost of the reagents, make this laboratory exercise appealing for instructional settings.

The students were also able to probe the new reaction acceleration that has not been previously reported in the literature. The results acquired by students were not pre-tested and provided a true experiment, rather than a "cookbook" laboratory, and in turn they demonstrated the first case of acceleration of a deprotection reaction and the first demonstrated case of reaction acceleration using EASI. The addition of studies on neutral spray droplets for reaction acceleration adds to the growing literature seen in the variety of accelerated methods such as Leidenfrost droplets [36], thin films [28], desorption electrospray ionization (DESI) [37] and other charged spray droplet systems [20]. Post-laboratory interviews showed that the students found this laboratory exercise both valuable and a worthwhile experiment.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ijms.2018.05.009.

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