International Journal of Mass Spectrometry 430 (2018) 98–103

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

Full Length Article

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

Patrick W. Fedick[a], Ryan M. Bain[a], Kinsey Bain[a], Tsdale F. Mehari[a], R. Graham Cooks[a,b,*]

[a] Department of Chemistry, Purdue University, West Lafayette, IN 47907, United States
[b] Center for Analytical Instrumentation Development, Purdue University, West Lafayette, IN 47907, United States

ARTICLE INFO

Article history:
Received 15 March 2018
Received in revised form 16 April 2018
Accepted 8 May 2018
Available online 9 May 2018

Keywords:
Organic synthesis
Laboratory instruction
Hands-on learning
Mass spectrometry
Reaction acceleration
Amino acids
Boc deprotection
Easy ambient sonic-spray ionization

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

* Corresponding author at: Department of Chemistry, Purdue University, West Lafayette, Indiana 47907, United States.
E-mail address: cooks@purdue.edu (R.G. Cooks).

https://doi.org/10.1016/j.ijms.2018.05.009
1387-3806/

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].

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

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.

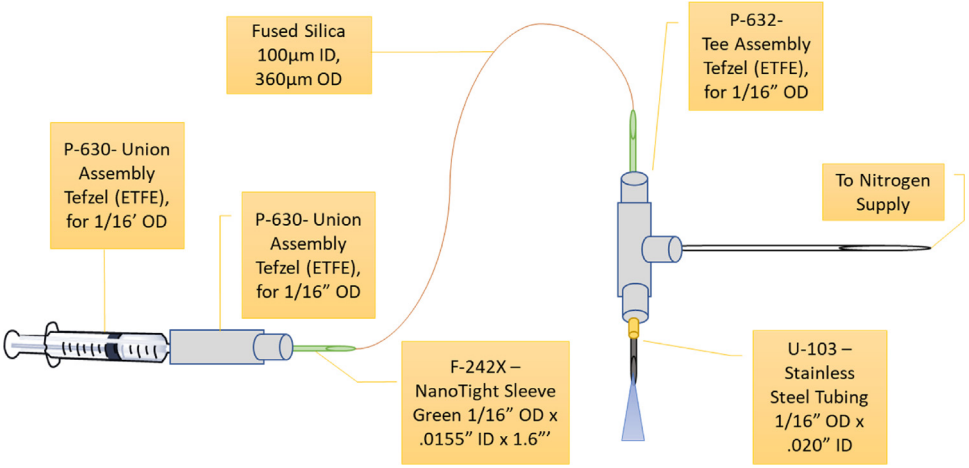

Fig. 1. Easy ambient sonic-spray ionization (EASI) droplet generation system consisting of a gastight syringe, fused silica lines, Teflon unions, nanotight sleeves and a stainless-steel capillary.