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Research Article

Aldol Reactions of Biorenewable Triacetic Acid Lactone Precursor Evaluated Using Desorption Electrospray Ionization Mass Spectrometry High-Throughput Experimentation and Validated by Continuous Flow Synthesis

H. Samuel Ewan, Shruti A. Biyani, Jaycie DiDomenico, David Logsdon, Tiago J. P. Sobreira, Larisa Avramova, R. Graham Cooks, and David H. Thompson*

Cite This: https://dx.doi.org/10.1021/acscombsci.0c00119

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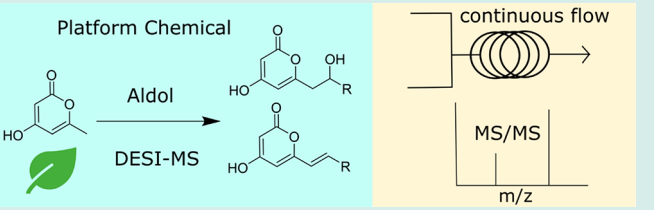
ABSTRACT: Desorption electrospray ionization-mass spectrometry (DESI-MS) was used as a high-throughput experimentation (HTE) tool to rapidly identify derivatives of the biobased platform molecule triacetic acid lactone (TAL). TAL is a platform molecule capable of conversion to a wide range of useful commodity chemicals, agrochemicals, and advanced pharmaceutical intermediates. In the present study, a diverse family of aldol reaction mixtures were prepared in high-density microtiter plates with a liquid handling robot, then printed with a pin tool onto a PTFE surface for analysis by DESI-MS. Our DESI-MS results indicate that aldol products of TAL were obtained for each substrate tested, in good agreement with previously reported TAL reactivity. These HTE experiments also revealed solvent-dependent reactivity trends that facilitated reaction scale up. Our findings suggest that DESI-MS analysis can rapidly inform the selection of optimal reaction conditions from a wide variety of conditions for scale up using continuous synthesis conditions.

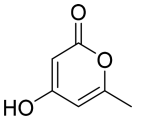


KEYWORDS: *triacetic acid lactone, platform chemical, high-throughput experiment, desorption electrospray ionization, MS/MS, aldol reaction*

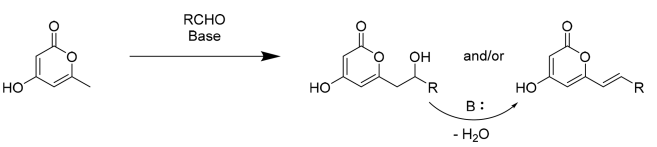
INTRODUCTION

Platform chemicals are versatile molecules that are converted to a wide array of value-added products with applications across various industries. The vast majority of such materials are sourced from petroleum, thus motivating the need to develop renewable and sustainable alternatives. Many biorenewable molecules have been explored for use as platform chemicals, including a wide variety of carbohydrate and furan precursors.[1−3] Glycerol is a carbohydrate that has been extensively derivatized into a number of value added products including polymers, coatings, surfactants, and synthetic intermediates.[4,5] 5-Hydroxymethylfurfural, a dehydration product of fructose, is another example of a biomass derived platform chemical that has been converted into a range of direct replacements for petrochemical products.[6,7] The polyketide class of natural products possess a highly diverse array of molecular architectures with alternating carbonyl-methylene motifs that make these compounds a promising family of precursors for the development of new platform chemicals.[3,8] Platform chemicals and biobased platforms will help us to achieve sustainable, clean, and energy efficient sources to meet the high industrial demand in the future. The goal of this work is to identify a rapid high-throughput

Scheme 1. Triacetic Acid Lactone (TAL)



Scheme 2. Aldol Reaction of TAL and Subsequent Elimination



Received: June 15, 2020
Revised: October 24, 2020

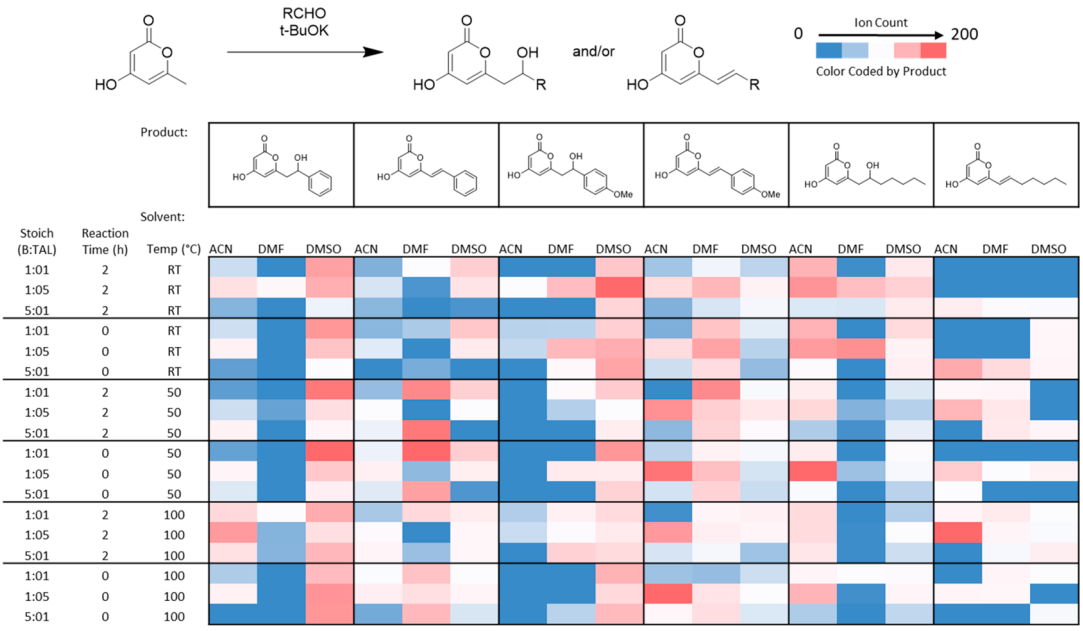
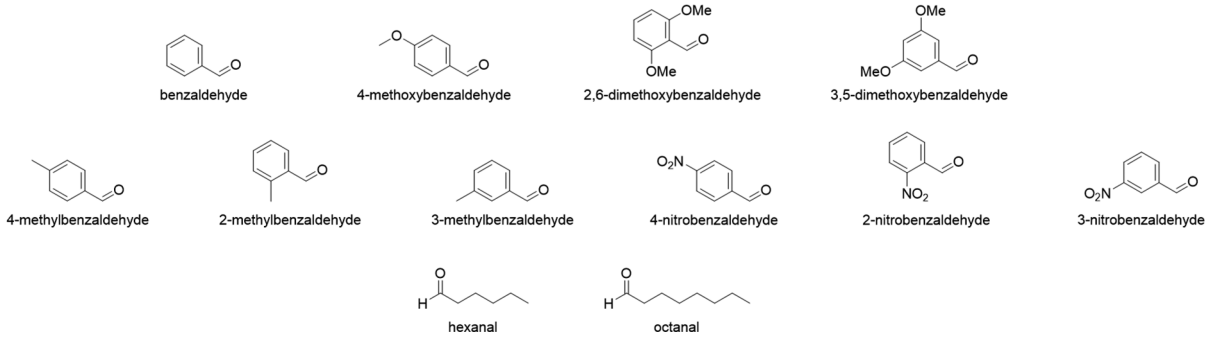


Figure 1. Heat map of aldol product signal intensities from aldol reactions of TAL with three substrates, three solvents, three stoichiometries, and three temperatures. Each cell is an average of 4 replicates with normalized MS intensities. Heat maps are color coded by product, separated by dark vertical lines in the table.

Scheme 3. Aldehyde Starting Materials Used in the TAL Reactivity HTE Study



approach for discovering and derivatizing a biobased platform chemical.

Triacetic acid lactone (TAL) is a polyketide (Scheme 1) that has been produced biosynthetically by Xie and co-workers[9] via fermentation from glucose using yeast or *E. coli* expressing genetically engineered polyketide synthase. TAL has been converted into a wide array of useful commodity chemicals, solvents, agrochemicals, and advanced pharmaceutical intermediates using different reaction classes including hydrolysis, hydration, amination, acylation, and aldol condensation.[8,10−17] Previous reports of aldol reactions with TAL showed that the aldol product may be produced using either two equivalents of base or a single equivalent of base if the TAL alcohol substituent has been protected. Use of more forcing conditions can promote elimination from that aldol product to form a conjugated alkene as shown in Scheme 2.[13−17] This study seeks to accelerate the process of developing platform chemicals using aldol reactions of TAL as a model system to expand the scope of products generated by this transformation in a diversity-oriented synthesis effort.

High-throughput experimentation (HTE) has been widely and successfully used in the drug discovery and development process.[18−26] We have recently reported the use of desorption electrospray ionization mass spectrometry (DESI-MS) as an HTE tool to accelerate the process of reaction optimization and upscaling.[27−31] DESI is an ambient ionization method that uses charged microdroplets of solvent to extract analytes from a surface prior to their introduction into a mass spectrometer.[28,32−34] This approach employs reaction arrays comprised of different reagent combinations, substrates and/or additive stoichiometries, solvent types, incubation times, and reaction temperatures using a Beckman Coulter Biomek i7 liquid handling robot to prepare the reaction mixtures in high-density well plates. After preparing the arrays and incubating for a given period of time, the reaction arrays are then printed onto a porous polytetrafluorethylene (PTFE) surface using a magnetic slotted-pin tool array that withdraws 50 nL per pin when dipped into wells containing the reaction mixture. The pin array is then pressed onto the PTFE surface, depositing a sample of the well mixture containing less than 1 μg of material per spot. This plate is then transferred to the DESI stage of the mass spectrometer, where a stream of charged solvent is sprayed onto the surface. The plate is moved beneath this solvent stream, scanning spot-by-spot over the entire reaction printed surface of the PTFE plate. As the stream passes over each reaction spot, the material on the surface is desorbed and

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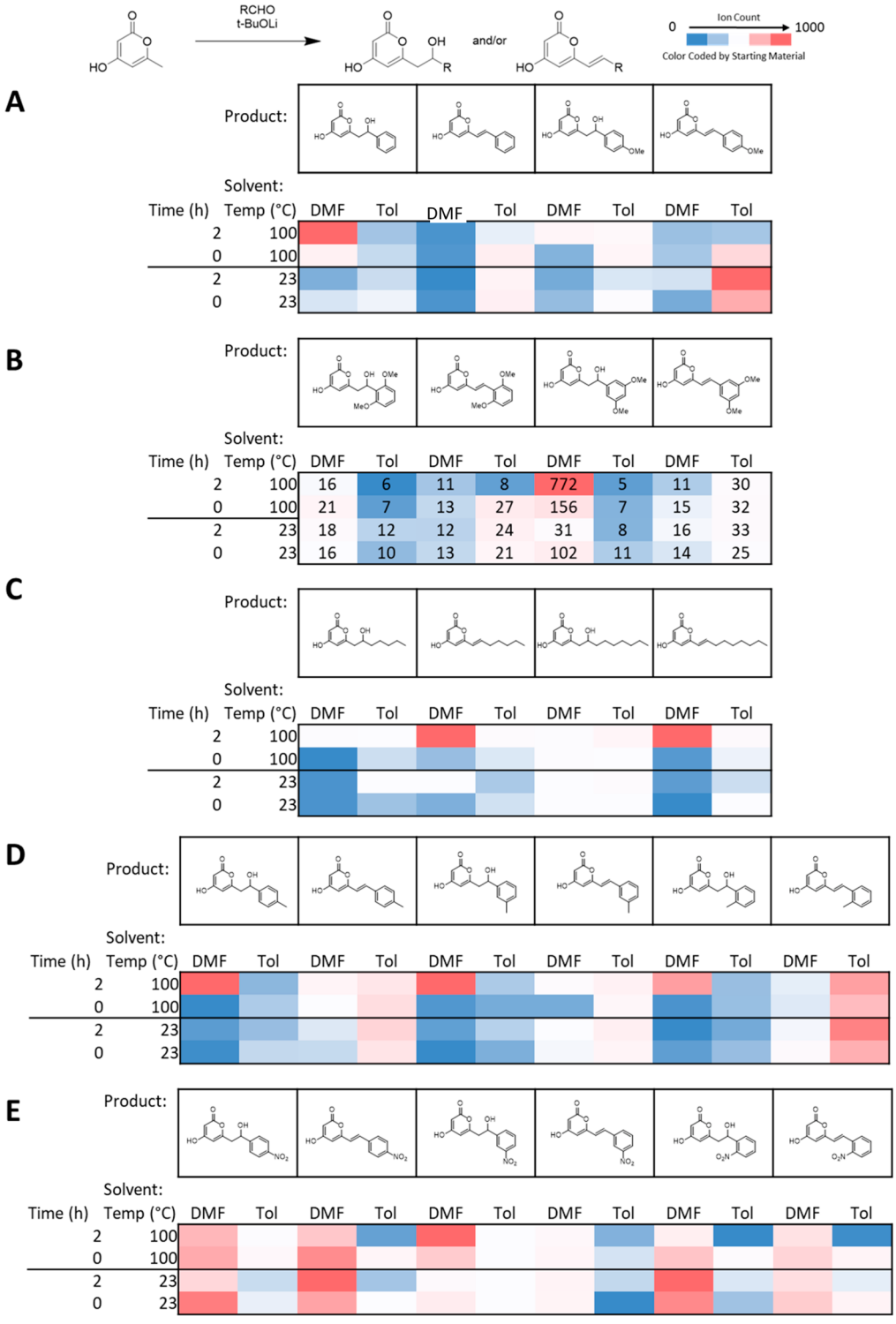

Figure 2. DESI-MS HTE heat map of the expanded aldol reaction set. Each cell is an average of 8 replicate reactions with normalized MS intensities. Heat maps are color coded by starting material type along the dark vertical lines in the tables. Ion intensity values are shown in the third heat map to more explicitly show the difference in substrate-based reactivity.

secondary droplets enter the MS inlet to be analyzed, producing a full MS spectrum for each point on the surface. These results may then be visualized as a product-ion intensity heat map, revealing successful or unsuccessful conditions to produce the product.[27,28] The most successful reactions revealed by this high-throughput screen may be validated by performing a scaled up reaction in a flow reactor analyzed by electrospray ionization MS to offer a quick secondary validation of the DESI-MS HTE results as our group has previously demonstrated.[35] In addition to validating the HTE results, the ease of automation and simplified optimization and scaling of microfluidic syntheses offers a simplified path forward for further development of derivatives of TAL identified by HTE.[36−38] This technology has the potential to greatly accelerate the development of platform chemicals such as TAL with a minimum input of time and resources,

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potentially identifying efficient pathways to renewable alternative precursors and end products more rapidly.

RESULTS AND DISCUSSION

First HTE Campaign. For the first experiment, aldol reactions of TAL were carried out using each possible

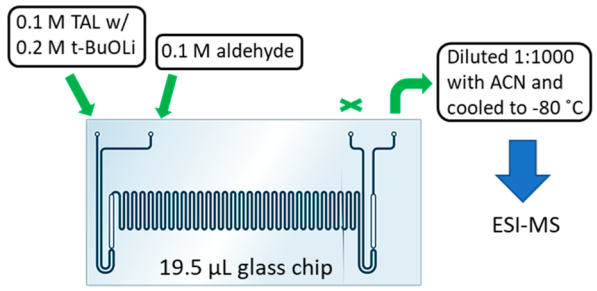

Figure 3. Microfluidic reactor schematic.

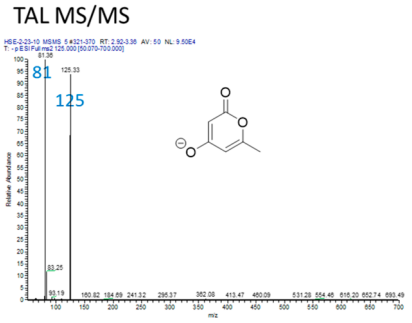

Figure 4. MS/MS spectrum of TAL.

combination of three different aldehyde substrates, solvents, stoichiometries, and reaction temperatures for a total of 81 unique reaction conditions. Each reaction was sampled just after reagent mixing and again after 2 h of heating. The reactions were prepared in duplicate wells, with each well printed twice to serve as pinning replicates. With 81 unique reactions sampled at two time points and deposited onto the PTFE plate as four replicates, a total of 648 reaction data points were produced in this experiment. The aldehydes used were benzaldehyde, 4-methoxybenzaldehyde, and hexanal at aldehyde/TAL stoichiometries of 5:1, 1:1, and 1:5. Two equivalents of potassium *t*-butoxide were used relative to TAL. Each reaction was prepared at 50 mM TAL concentration with at least 300 μL of solution in each well. The i7 liquid handling robot was used to prepare reaction mixtures in 96 well plates with glass inserts that were sealed with PTFE lined silicone rubber mats and heated on home-built constant temperature plates. Each reaction was incubated for 2 h at 20 °C, 50 °C, or 100 °C, and two 30 μL samples were withdrawn before and after the incubation period. Upon withdrawing these aliquots from the 96 well plates, they were distributed into 384 well plates to be pinned with the 384 pin tool, thus creating an additional "pinning" replicate. Reaction arrays were then printed onto glass supported PTFE for DESI-MS analysis. The raw MS data was processed using in-house software and visualized as a heat map as shown in Figure 1.

The heat map shows hits for both the aldol and elimination products for each of the three aldehyde types. Some reactivity trends also become apparent from examination of the heat maps. In the case of the two aromatic aldehydes, benzaldehyde and 4-methoxybenzaldehyde, DMSO appeared to give better conversion to the alcohol product, whereas DMF appeared to enable greater elimination product formation. These reactions appeared to take place at 20 °C as well as at elevated temperatures. For the aliphatic aldehyde hexanal, ACN appeared to be the preferred solvent for generating the aldol product, with the alkene product detected only at higher reaction temperatures. While varying substrate, solvent, and temperature appeared to affect the outcome of the reactions, stoichiometry seemed to have little discernible effect.

Second HTE Campaign. After this first set of transformations, the substrate scope was expanded to include aldehydes with varying steric and electronic characteristics (Scheme 3). Reaction arrays were prepared and sampled before and after the 2-h heating period as described in the previous experiment. For this experiment, lithium *t*-butoxide (two equivalents) was used as a base instead of potassium *t*-butoxide due to the greater solubility of the lithium species in the solvents used in this reaction series. This experiment was also limited to two solvents, two temperatures, and a 1:1 TAL/aldehyde stoichiometric ratio since the previous stoichiometry experiment revealed that this was not an important variable to explore. The solvents selected were DMF, a polar solvent that produced successful reactions in the previous screen, and toluene, one of the least polar solvents. During the DESI-MS analysis stage, the negative ionization mode was used, as opposed to positive in the previous experiment, since the TAL derivatives were more easily detected in negative ion mode. With this simplified array, the experiment explored 48 unique reaction conditions with 8 replicates each, measured at 2 time points for a total of 768 data points.

The most readily apparent trend is the consistent success of reactions carried out in DMF. Further, most of these reactions in DMF were more successful with the application of heat (Figure 2A–E). Notably, the 2,6-dimethoxybenzaldehyde precursor gave lower conversion than the 3,5-dimethoxybenzaldehyde starting material, indicating that differences in reactivity based on steric factors may be detected with this method.

Microfluidic Validation of Selected HTE Hits. In order to validate the results of the DESI-MS experiment and build confidence in the capacity of these HTE experiments to predict upscaled reaction outcomes, several of the reactions were conducted under continuous flow conditions in a microfluidic reactor. A Chemtrix S1 system fitted with a 19.5 μL glass reactor chip with staggered-oriented-ridge (SOR) in line mixers (Figure 3) was used for this experiment. A solution of premixed 0.1 M TAL and 0.2 M lithium *t*-butoxide in DMF was flowed into one inlet to engage a 0.1 M aldehyde solution. A subset of the aldehydes evaluated in the DESI-MS HTE experiment were selected, specifically 4-methoxybenzaldehyde, hexanal, 2,6-dimethoxybenzaldehyde, and 3,5-dimethoxybenzaldehyde. Residence times of 0.5, 1, 2, 5, and 10 min and temperatures of 20, 100, and 200 °C were investigated. After emerging from the reactor, each sample was diluted 1:1000 into ACN and cooled to −80 °C until analysis by electrospray ionization-MS (ESI-MS).

The aldehydes tested in the flow reaction screen each showed at least one condition that generated a product m/z by ESI-MS analysis. For further confirmation of product identity, MS/MS spectra were acquired for the product peaks and compared to the MS/MS of TAL (Figure 4) that showed a distinctive fragment at m/z 81 in negative ion mode.

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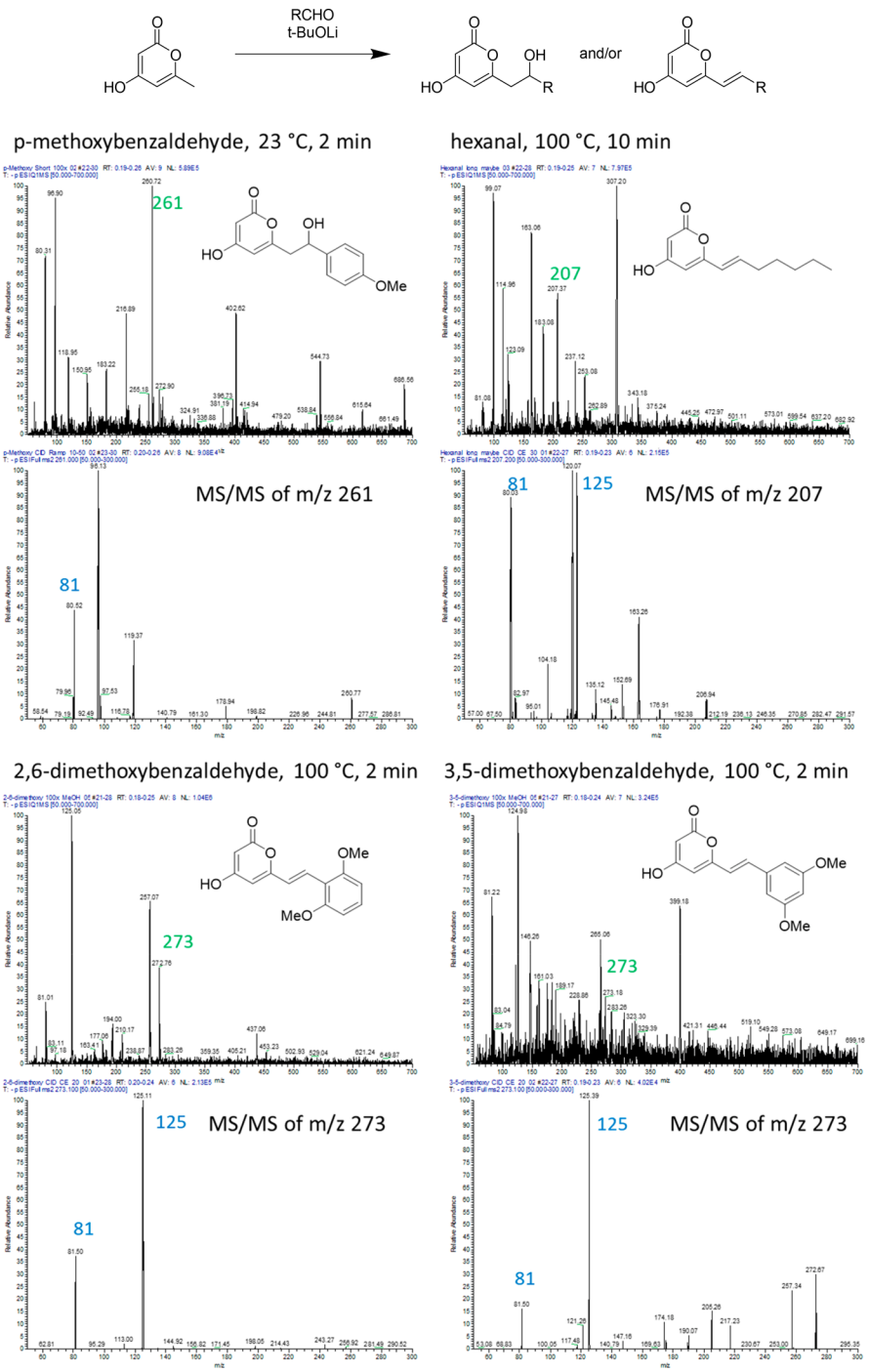

Figure 5. MS and MS/MS analysis of microfluidic reactions corresponding to DESI-MS HTE results.

For 4-methoxybenzaldehyde, the strongest product signal detected from the flow reaction screen was observed with a 2 min residence time at a temperature of 20 °C. The peak at m/z 261 corresponds to the M − H peak in the negative MS mode (Figure 5). MS/MS of this m/z 261 product gave a fragment at m/z 81, suggesting a structural similarity to TAL. The hexanal reactions showed evidence of the elimination product at m/z 207 but not of the aldol product. The greatest yield of the elimination product of hexanal and TAL was observed at a residence time of 10 min at 100 °C. This was the only case where the elimination product was observed in the set of microfluidic experiments. MS/MS analysis of the m/z 207 product revealed fragments at m/z 125 and m/z 81, suggesting that the m/z 207 species is the elimination product of the aldol formed by the reaction of hexanal and TAL. Peaks at m/z 273 were observed in the aldol reactions of both 2,6- and 3,5-dimethoxybenzaldehyde, indicating the formation of the aldol adduct. Both had the highest product conversion at 2 min residence times at 100 °C. Further, the MS/MS data for both of the dimethoxybenzaldehyde substrates displayed fragments at m/z 125 and m/z 81. Counterintuitively, 2,6-dimethoxybenzaldehyde had a slightly higher conversion to the alcohol product than 3,5-dimethoxybenzaldehyde; however, the difference in the flow reaction outcomes were less marked than in DESI-MS HTE.

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Identification of Possible Regioisomers by LC-MS and MS/MS. Since there is a possibility of formation of regioisomer by substitution at the C-3, C-5, or 6α position of TAL, we investigated the products of reaction of TAL with benzaldehyde by LC-MS and MS/MS. We analyzed the 384 well-plate reaction mixture by LC-MS and performed the synthesis under batch conditions to identify the regioisomer by tandem mass spectrometry (ESI-MS/MS). Our LC-MS analysis indicated only a single peak for the alcohol adduct m/z 233.2 in positive ion mode, indicating the formation of a single isomer. The fragmentation pattern of MS/MS highlighted the neutral loss of water supporting the formation of 6α derived product as proposed in Scheme 2 (see the Supporting Information for detailed LC-MS and MS/MS fragmentation).

CONCLUSION

We have demonstrated the use of DESI-MS analysis as an HTE tool for evaluating aldol reactions with triacetic acid lactone. Using robotically prepared surface arrays of aldol reactions, the DESI-MS experiments were validated by corresponding microfluidic reactions analyzed by ESI-MS and MS/MS to rapidly identify aldol derivatives of TAL. DESI-MS HTE showed hits for all aldehyde substrates tested and provided useful information to guide reaction scale up by continuous flow reaction with respect to solvent and temperature reactivity trends. This technology represents an opportunity to accelerate the process of platform chemical transformation so that biorenewable replacements as well as novel chemical building blocks may be more quickly developed.

MATERIALS AND METHODS

General Procedure for DESI-MS Experiments. The DESI-MS experiments were conducted in the manner previously described by Wleklinski and co-workers.[28] A Biomek i7 liquid handling robot (Beckman Coulter) was used to distribute reaction mixtures into master well plates (either 96 or 384 well plates, depending on the scope of the experiment). For heated reactions, the liquid handling robot was used to distribute reaction mixtures into aluminum well plates fitted with glass vials (Analytical Sales & Services, Inc.). These heated master plates were prepared and sealed with a PFA film and two silicone rubber mats before heating. Each reaction vial was prepared at 50 mM and contained at least 300 μL of reaction mixture. After allowing the plates to cool, the reaction mixtures were transferred to secondary plates prior to printing onto DESI surfaces. A magnetic pin tool (V&P Scientific, Inc.) was interfaced with pod1 of the liquid handling robot and used to transfer 50 nL volumes of reaction mixtures from the master well plate to the porous PTFE surface for DESI-MS. A commercial DESI source (Prosolia, Inc.) and a Thermo LTQ linear ion trap were utilized to execute the DESI-MS experiment. Experiments were conducted in positive-ion mode (m/z 50−500) with pure methanol as the spray solvent (2.5 μL/min). Parameters for the mass spectrometer and speed of the DESI stage were optimized previously.[28] In-house software was used to process the data and generate spreadsheets from which heat maps were prepared.

General Protocol for Continuous-Flow Experiments. All microfluidic reactions were carried out using a Chemtrix Labtrix S1 system equipped with 3227 glass reactor chips with a volume of 19.5 μL. A solution of 0.05 M TAL and 0.1 M lithium *tert*-butoxide together, and another of 0.05 M aldehyde in DMF were prepared. Syringes were loaded with each of these solutions and positioned on the first two inlets of the Chemtrix 3227 chip. The reactants were engaged with 1 min, 2 min, 5 min, and 10 min residence times at temperatures of 20, 100, and 200 °C. Samples were collected and immediately diluted 1:1000 in ACN and stored at −80 °C prior to analysis.

General Procedure for ESI-MS Analysis. Mass spectral analysis was performed for each flow and batch reaction sample using a Thermo TSQ triple quadrupole mass spectrometer (Thermo Fisher Scientific) equipped with an autosampler and electrospray (ESI) ionization. Reaction samples were diluted 1:1000 into ACN upon collection and cooled to −80 °C to quench the reactions before warming to room temperature immediately prior to analysis. The distance between the tip of the spray emitter and the ion transfer capillary to the MS was kept constant at ∼1.5 cm. Experiments were performed using a Thermo-Fisher HESI-II probe and Ion Max ion source. A spray voltage of 3.5 kV was used for all analyses.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acscombsci.0c00119.

Detailed LC-MS protocol, MS/MS, and fragmentation (PDF)

AUTHOR INFORMATION

Corresponding Author

David H. Thompson − *Department of Chemistry, Purdue University, Purdue University Center for Cancer Research, Multidisciplinary Cancer Research Facility, Bindley Bioscience Center, West Lafayette, Indiana 47907, United States;* orcid.org/0000-0002-0746-1526; Email: davethom@purdue.edu

Authors

H. Samuel Ewan − *Department of Chemistry, Purdue University, Purdue University Center for Cancer Research, Multidisciplinary Cancer Research Facility, Bindley Bioscience Center, West Lafayette, Indiana 47907, United States*

Shruti A. Biyani − *Department of Chemistry, Purdue University, Purdue University Center for Cancer Research, Multidisciplinary Cancer Research Facility, Bindley Bioscience Center, West Lafayette, Indiana 47907, United States;* orcid.org/0000-0003-2756-3906

Jaycie DiDomenico − *Department of Chemistry, Purdue University, Purdue University Center for Cancer Research, Multidisciplinary Cancer Research Facility, Bindley Bioscience Center, West Lafayette, Indiana 47907, United States*

David Logsdon − *Department of Chemistry, Purdue University, Purdue University Center for Cancer Research, Multidisciplinary Cancer Research Facility, Bindley Bioscience Center, West Lafayette, Indiana 47907, United States*

Tiago J. P. Sobreira − *Department of Chemistry, Purdue University, Purdue University Center for Cancer Research, Multidisciplinary Cancer Research Facility, Bindley*

https://dx.doi.org/10.1021/acscombsci.0c00119
ACS Comb. Sci. XXXX, XXX, XXX−XXX

Bioscience Center, West Lafayette, Indiana 47907, United States

Larisa Avramova − *Department of Chemistry, Purdue University, Purdue University Center for Cancer Research, Multidisciplinary Cancer Research Facility, Bindley Bioscience Center, West Lafayette, Indiana 47907, United States*

R. Graham Cooks − *Department of Chemistry, Purdue University, Purdue University Center for Cancer Research, Multidisciplinary Cancer Research Facility, Bindley Bioscience Center, West Lafayette, Indiana 47907, United States;* orcid.org/0000-0002-9581-9603

Complete contact information is available at: https://pubs.acs.org/10.1021/acscombsci.0c00119

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors gratefully acknowledge the support of DARPA Award No. W911NF-16-2-0020 and NIH Grant P30 CA023168 of the Purdue University Center for Cancer Research (NMR, MS, and MPF Shared Resources).

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https://dx.doi.org/10.1021/acscombsci.0c00119
ACS Comb. Sci. XXXX, XXX, XXX−XXX