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Microgram-Scale Testing of Reaction Conditions in Solution Using Nanoliter Plugs in Microfluidics with Detection by MALDI-MS

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This paper describes a microfluidic system to screen and optimize organic reaction conditions on a submicrogram scale. Optimization of reaction conditions is required to achieve high efficiency and selectivity in organic reactions. Combinatorial methods¹ and high-throughput screening² are powerful tools for optimization. To perform solution-phase synthesis, typical microtiter plates or reaction blocks for parallel synthesis run reactions on the scale of mL/reaction¹ and are less applicable to precious substrates (e.g., products of long synthetic sequences and natural products that can be isolated only in small quantities). To address this problem, one approach used arrayed micro-wells in combination with a robotic liquid sampler on the scale of ~125 nL per reaction.³ To reduce the use of robotics and to minimize evaporation, others used microchannels^{4–6} to perform reactions, including synthesis of pyrazoles with UV detection (5 μ L per reaction)⁶ and optimization of glycosylation conditions⁵ (~2 mg reagent per reaction).

Here, we report a screening method that consumes substrates on the scale of less than 1 μ g per reaction. The system uses discrete droplets (plugs)⁷ as microreactors⁸ separated and transported by a continuous phase of a fluorinated carrier fluid. Such approach is not limited to microfluidics—fluorinated fluids were used previously to segment samples in NMR⁹ and PCR¹⁰ in tubes to prevent dispersion of sample solutions. Previously, we demonstrated the use of a microfabricated PDMS plug-based microfluidic system to perform assays and crystallization experiments in aqueous solutions with optical detection.¹¹ Here, we developed an approach that does not require microfabrication of microfluidic devices,¹² is applicable to synthetic reactions in organic solvents, and uses detection by MALDI-MS.

The system consisted of three components: preformed cartridge^{11,13} of reagent plugs, a PEEK Tee, and a receiving tubing (Figure 1). A cartridge is an array of discrete plugs surrounded by fluorinated carrier fluid; each plug is composed of a solution of a different reagent. The cartridge was prepared by serially aspirating the reagents into a piece of Teflon tubing prefilled with carrier fluid. A commercially available PEEK Tee connected the cartridge and the inlet tubing, containing as little as a submicroliter volume of a solution of the substrate. Fluorinated carrier fluid (FC-70) was used to fill the two syringes (Figure 1) and the connecting tubing, enabling no-loss manipulation of submicroliter volumes of solutions. FC-70 has low miscibility with organic reagents and reasonably low viscosity (Supporting Information). To perform the reactions, the flow was induced with the two syringes, and the reagent plugs were sequentially merged with the substrate solution. After all resulting plugs flowed out of the Tee into the receiving tubing, the flow was stopped, and the receiving tubing was sealed. After incubation,

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 Supporting Information Available: Materials and methods, control experiments, and additional MALDI-MS data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

the reaction plugs were deposited onto a MALDI plate, evaporated, and then a solution of MALDI matrix was deposited over, dried, and analyzed by MALDI-MS.^{2,14,15} We were helped by work on single-neuron analysis,¹⁶ where ~150 nL droplets were used. Here, the low surface tension of the fluorinated carrier fluid facilitated deposition of ~30 nL droplets.

Control experiments were performed to characterize merging. Relative volumetric flow rates of substrate and reagent solutions controlled the ratio in which they combined. Reagent plugs were typically 15 nL in volume, and each combined with 11 nL substrate solution during merging (established by high-resolution digital microphotographs). To avoid potential cross-contamination between neighboring plugs during merging, we introduced one long “blank” solvent plug between every two reagents.¹¹ About 22 nL of substrate solution was deposited into each blank plug. Fluorescence measurement showed that the cross-contamination was below 1% (Supporting Information).

To test this screening system, we used as an example the deacetylation (Scheme 1) of ouabain hexaacetate (Ac₆-OUA). We are interested in derivatives of ouabain (OUA) because it is a poisonous cardiac glycoside and is extensively used in studies of neurons¹⁷ to block the sodium pump (Na–K–ATPase). Selective deacetylation of Ac₆-OUA has been reported only to the ouabain triacetate (Ac₃-OUA) in 22% yield.¹⁸

We envisioned that screening for optimal conditions would consist of two steps—screening a large number of reagents to evaluate reactivity rapidly, and then repeating the screening with a narrower set of reagents while varying reaction conditions (time, solvents, and concentrations). To determine the best reagents to prepare Ac₃-OUA, we merged six cartridges containing 44 reagents with 0.01 M Ac₆-OUA solution in 1:1 dioxane:ethanol, incubated the reaction plugs for 1 h at 18 °C, deposited the plugs to the MALDI-plate, and quenched the reactions by adding acidic matrix solution. To evaluate the reaction efficiency by MALDI-MS, we compared the fraction of peak area (*A*) of Ac₃-OUA (defined as $A_{Ac_3-OUA} / \sum A_{\text{all OUA derivatives}}$) for each reagent (Figure 2). MALDI-MS can be used to characterize reactions quantitatively or semi-quantitatively.^{2,14,15} We confirmed that there was a strong correlation between the fraction of peak area in MALDI-MS of the compound and its actual fraction in the sample. Measurements among samples with different salt additives were reproducible (Supporting Information).

Consuming ~20 μg of the substrate (~2 μL of solution), we established that inorganic bases showed the highest reactivity for hydrolysis, followed by several organic amines, and all the Lewis acids and lipases tested showed very low reactivity. On the basis of this screening, we chose reagent 39 (0.06 M Na₂CO₃ in 1:2 H₂O: methanol) for 0.1 mmol scale-up reaction. Using 83.7 mg of Ac₆-OUA, we obtained 60.3 mg of Ac₃-OUA (85% isolated yield) (Scheme 1).

To optimize reaction conditions on a submicrogram scale, we followed the reaction progress with amines or inorganic bases as the reagents (Figure 3). Regioisomers of ouabain tetraacetate (Ac₄-OUA) and ouabain pentaacetate (Ac₅-OUA) are unstable intermediates in hydrolysis of Ac₆-OUA, and their syntheses have not been reported. By quenching reactions at different times, we were able to follow the reaction course in solvents, including DMF, dioxane, acetonitrile, methanol, and water. Using the optimized conditions, we performed 0.1 mmol (83.7 mg) scale reactions and obtained Ac₅-OUAs and Ac₄-OUAs, with isolated yields of 39% (25% of major regioisomer) and 47% (19% of major regioisomer), respectively (Scheme 1).

A potential problem of microfluidic approaches is large overhead consumption of substrate during interfacing of the chip with the macroscopic world—while an experiment itself may consume only a few microliters of solution, a much larger volume of solution may be lost to fill the connecting lines and delivery channels. To demonstrate the no-loss handling of substrate

with this system from start to finish, we dissolved 7 μg of $\text{Ac}_6\text{-OUA}$ in 0.8 μL of DMF, merged the solution with 14 reagent plugs, and obtained MALDI-MS data consistent with other screens.

This solution-phase screening method can be extended to multistep synthesis¹⁹ and generates little waste. It will be complementary to the methodologies using substrates attached to solid surfaces.^{1,15} To optimize this system, we are developing a merging method that will eliminate cross-contamination without introducing blank solvent plugs. Performing reactions under anhydrous and/or anaerobic conditions was not demonstrated here, but it should be possible to use this compact system in a glovebox. Screening reactions at elevated temperatures requires further study to evaluate the dissolution of reagents in carrier fluid. As a preliminary step, we performed several deacetylation reactions at 40 °C and obtained data consistent with the expected reactivity of reagents. In this study, we used MALDI-MS to follow the reaction progress. It is semiquantitative but cannot easily distinguish between isomers. Integrating micro separation and characterization methods (e.g., capillary-LC or microcoil-NMR⁹) into this system will improve it further. This simple system for performing submicrogram, nanoliter scale reactions should become useful for optimizing reactions of precious substrates over a large chemical space.

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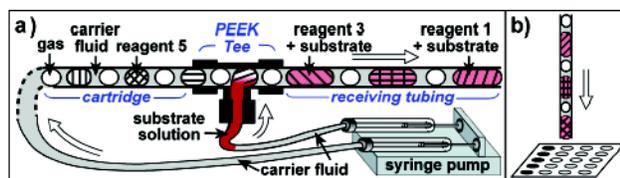


Figure 1. Workflow of the screening system. (a) Serial merging of the substrate stream with reagent plugs from a cartridge. Blank solvent plugs are not shown. (b) After incubation, the reaction plugs are deposited onto a sample plate for MALDI-MS.

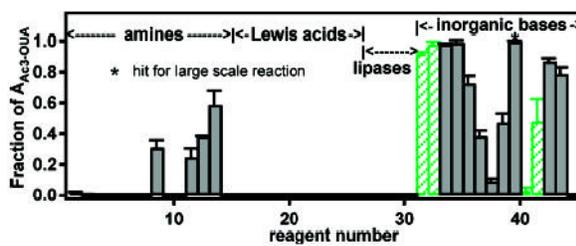


Figure 2.

Testing hydrolysis of Ac_6 -OUA to Ac_3 -OUA with 44 reagents. Fraction of A_{Ac_3-OUA} was the ratio of the peak area of Ac_3 -OUA in MALDI-MS over the total area of all the peaks that contain derivatives of OUA. Dashed (versus gray) bars indicate excessively (versus insufficiently) hydrolyzed products as major impurities. The error bars are one standard deviation based on three parallel experiments. The identity of reagents is given in Supporting Information.

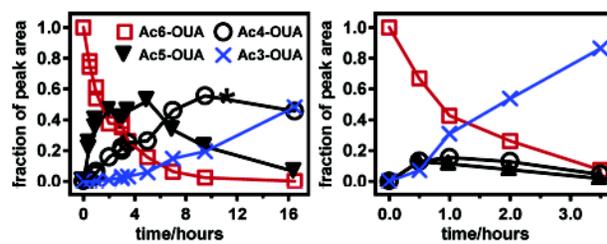
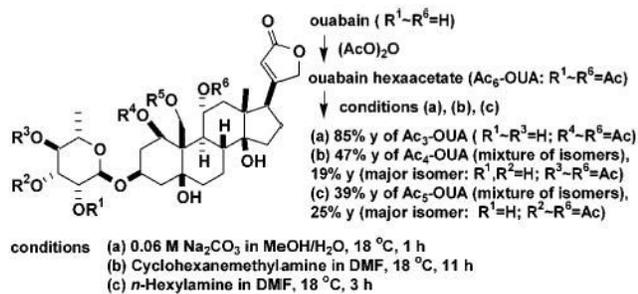


Figure 3.

The relative concentrations of species (approximated as fraction of peak areas in MALDI-MS) during the hydrolysis of Ac₆-OUA at 18 °C. (Left): reagent = cyclohexanemethylamine, substrate = 0.01 M Ac₆-OUA in DMF. Asterisk (*) indicates the reaction time used in the large-scale reaction. (Right) reagent = 0.3 M 1:2 NaOPh:PhOH in 2:1 methanol:H₂O, substrate = 0.01 M Ac₆-OUA in 1:1 ethanol:dioxane.



Scheme 1.