COMBINATORIAL SYNTHESIS OF PEPTIDOMIMETICS USING DIGITAL MICROFLUIDICS

Mais J. Jebrail[1], Naila Assem[2], Jared M. Mudrik[2], Michael D. M. Dryden[2], Kaixiang Lin[2], Andrei K. Yudin[2], and Aaron R. Wheeler[2]

[1]Department of Biotechnology & Bioengineering, Sandia National Laboratories, USA
[2]Department of Chemistry, University of Toronto, CANADA

ABSTRACT

A microfluidic technique for combinatorial chemical synthesis of peptidomimetics has been developed. The new 32-step method is fast, automated and includes an integrated magnetic separation of inorganic catalysts from reaction products. This proof-of-concept study should lead to methods for generating libraries of compounds suitable for screening for bioactivity.

KEYWORDS

Digital microfluidics, combinatorial synthesis, peptidomimetics, therapeutics discovery

INTRODUCTION

The miniaturization of synthesis offers advantages such as high-throughput operations and faster reactions [1]. Recently, digital microfluidics (DMF) has become popular for applications in chemical synthesis [2,3]. In DMF, droplets of reagents (nL–µL), each serving as a discrete microreactor, are manipulated by applying a series of electrical potentials to an array of electrodes coated with a hydrophobic insulator [4]. The DMF format shares many of the advantages of microchannels, with added benefits of inert device materials, control over reagents without pumps, valves, or tubing, and facile control of both solids and liquids (i.e., there are no channels to clog). Here, we report the first digital microfluidic method for combinatorial chemical synthesis, applied to the synthesis of peptidomimetics and related products. The new method is fast, automated and includes an integrated magnetic separation of inorganic catalysts from reaction products [5].

EXPERIMENTAL

DMF devices were fabricated in the University of Toronto Emerging Communications Technology Institute (ECTI) cleanroom facility as described in detail elsewhere [6].

For analysis by mass spectrometry, ~2.3 mM solutions of reaction products formed by digital microfluidics were dissolved in 70 µL aliquots of methanol containing 0.1% formic acid. These solutions were injected into a 1200 series quadrupole mass spectrometer (Agilent, Santa Clara, CA) operating in positive ion mode. The samples were delivered at a flow rate of 0.5 mL min^{-1}, with an applied voltage of 50 V and capillary temperature of 100ºC.

RESULTS AND DISCUSSION

The starting reagents for the synthesis reported here, shown in Figure 1, are two thioacid peptides (***A*** $_{1,2}$) and two NH aziridine terminated amino acids (***B*** $_{1,2}$). We reacted ***A*** $_{1,2}$ and ***B*** $_{1,2}$ to form four peptidomimetic products (***A*** $_i$-***B*** $_j$) as shown in Table 1.

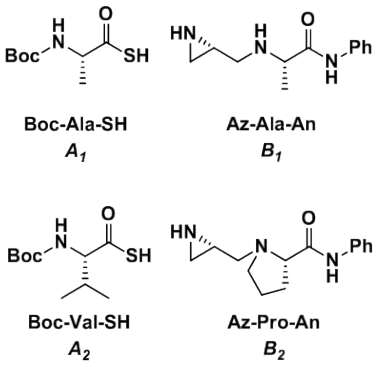
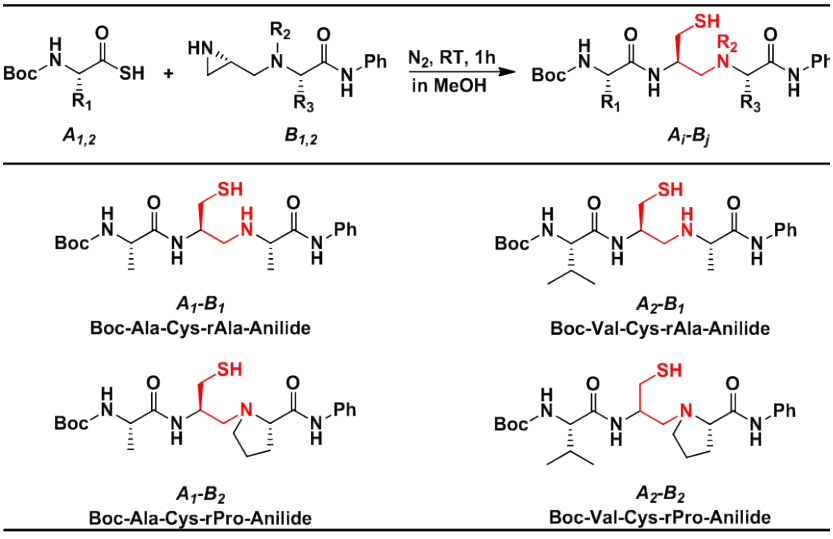


Figure 1: *Starting materials used for combinatorial peptidomimetic synthesis: thioacidpeptides (*$A_{1,2}$*) and NH aziridine terminated amino acids (*$B_{1,2}$*).*

Table 1*. Reaction scheme (top) and products (bottom) for the combinatorial synthesis of peptidomimetics.*



The device used for this work, shown in Figure 2a, has an array of actuation electrodes connecting a series of reservoirs containing various reagents. Figure 2b demonstrates the full protocol required for synthesis of peptidomimetics. First, four droplets containing the thioacid peptide substrates (two each of $\boldsymbol{A_1}$ and $\boldsymbol{A_2}$) were dispensed from their respective reservoirs. Second, four droplets containing the NH aziridine terminated amino acid substrates (two each of $\boldsymbol{B_1}$ and $\boldsymbol{B_2}$) were dispensed. Third, the droplets were combinatorially merged, mixed, and incubated for 1 h. Fourth, the solvent was evaporated to isolate the peptidomimetic products.

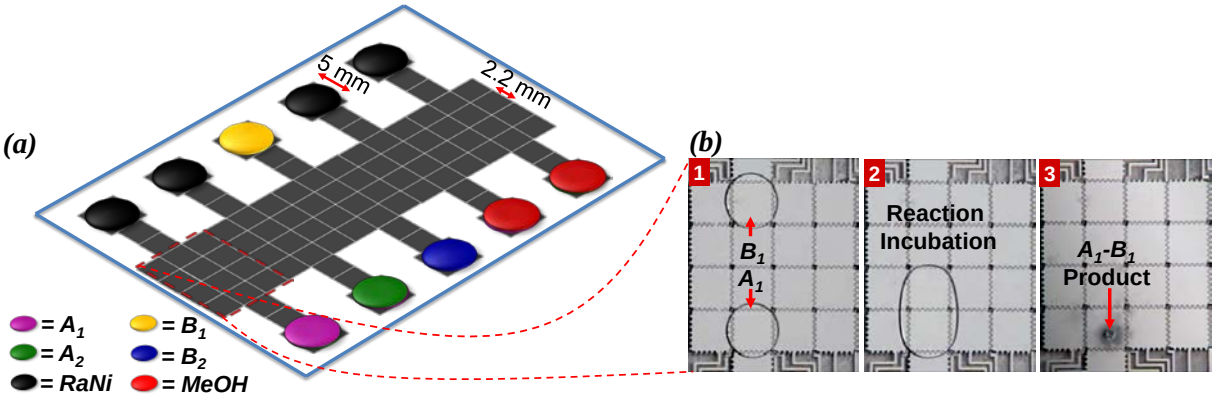


Figure 2: ***(a)*** *Schematic of the digital microfluidic device used for combinatorial peptidomimetic ligation.* ***(b)*** *Sequence of frames from a movie illustrating digital microfluidic-based ligation. In frames 1 and 2, droplets (900 nL each) containing thioacid peptides A_1 and NH aziridine terminated amino acid B_1 were dispensed from their respective reservoirs, merged, mixed, and reacted in a miniaturized glovebox (not shown) at room temperature for 1 h. Finally, in frame 3, peptidomimetic product A_1-B_1 was isolated by allowing the solvent to evaporate (room temperature, ~15 min).*

Mass spectrometry (MS) was used to evaluate the efficacy of synthesis by the digital microfluidic method. Figure 3a,b shows representative mass spectra of $\boldsymbol{A_1}$-$\boldsymbol{B_1}$ and $\boldsymbol{A_1}$-$\boldsymbol{B_2}$, with peaks at m/z 425 and m/z 451, respectively. The MS spectra for products $\boldsymbol{A_2}$-$\boldsymbol{B_1}$ and $\boldsymbol{A_2}$-$\boldsymbol{B_2}$ were also obtained, but data not shown. Peptidomimetic products were further modified to form desulfurized analogues as illustrated in Figure 4. First, four droplets of solvent (methanol) were dispensed and delivered to the solid products for dissolution. Second, four droplets containing Raney Nickel (RaNi) catalyst were dispensed, merged, and mixed with the droplets containing the peptidomimetics, and the reactions were allowed to incubate for 15 min. Third, the catalyst was immobilized using a magnet and the product droplets were driven away. Fourth, the solvent was evaporated to isolate the desulfurized products. MS spectra (Fig. 3c,d) of desulfurized $\boldsymbol{A_1}$-$\boldsymbol{B_1}$ (m/z 393) and $\boldsymbol{A_1}$-$\boldsymbol{B_2}$ (m/z 419) demonstrated that the reaction was driven to completion, as there were no peaks representative of the starting materials. This trend was also observed for the other desulfurized peptidomimetic compounds (data not shown).

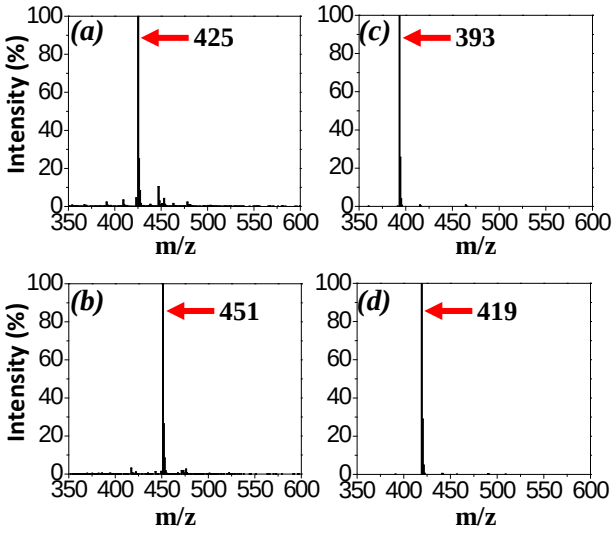


Figure 3: *Mass spectra of peptidomimetic products* ***(a)*** *A_1-B_1 and* ***(b)*** *A_1-B_2, and desulfurized products* ***(c)*** *A_1-B_1 and* ***(d)*** *A_1-B_2 synthesized by digital microfluidics.*

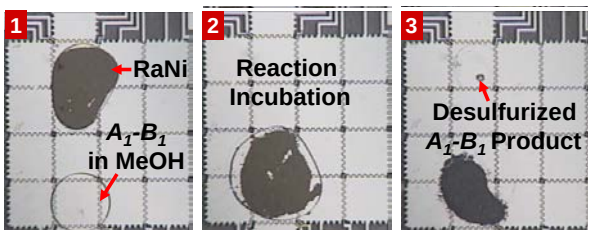


Figure 4: *Sequence of frames from a movie illustrating the steps of desulfurization with Raney Nickel (RaNi) on a digital microfluidic device. In frames 1 and 2, peptidomimetic products are solubilized in methanol, merged with droplets (4.5 μL) of RaNi, and then incubated for 15 min at room temperature. In frame 3, desulfurized products were isolated by immobilizing the Ni with a magnet (not shown) and driving the reaction mixture away to evaporate Finally, in frame 3, peptidomimetic product A_1-B_1 was isolated by allowing the solvent to evaporate (room temperature, ~15 min).*

The new digital microfluidic method has several advantages relative to conventional techniques. This method delivers faster reactions and represents a >1000-fold reduction in reagent use relative to lab-scale methods (microliters relative to milliliters). In addition, the digital microfluidic method has the advantage of simplicity – no stir bars, stir plates, or rotary evaporators were required.

A critical step for many synthetic protocols (including those described here) is the removal of solvent, collection of some of the intermediate products for analysis, and re-dissolution for further processing. These steps can be challenging to implement in enclosed microchannels, but digital microfluidics is well-suited for forming precipitates and dissolving them [2]. Moreover, the reconfigurability of digital microfluidics allows for flexible solvent metering. In preliminary work, we found that 900 nL droplets of solvent were adequate to dissolve each of the peptidomimetic solids formed here, but in future experiments, much larger volumes (up to hundreds of microliters) could be used depending on the solubility of each compound.

A significant novelty in the methods described here is the use of magnetic forces to separate a RaNi catalyst (composed of grains of nickel-aluminium alloy) from desulfurized products. In this process, a magnet was used to immobilize RaNi to the device surface and supernatant containing products was driven away using digital microfluidics (Fig. 4). Magnetically controlled catalysts are currently attracting attention in the chemical synthesis community [7] because of the ease of recovery and reuse of catalyst materials. As far as we are aware, the methods reported here represent the first combination of magnetic isolation of inorganic catalysts implemented by microfluidics (of any format). We propose that this represents an important step forward for the field, as there is great potential for the development of rapid, automated synthesis with reusable magnetic catalytic materials (e.g., MagTrieveTM catalysts). This proof-of-concept is likely just the beginning; by increasing device footprint and/or operating multiple devices in parallel we propose that it will be straightforward to synthesize tens of products in parallel.

CONCLUSIONS

In summary, we report a new microfluidic technique for combinatorial synthesis, applied to formation of peptidomimetics and their desulfurized analogues. This method strengthens the prospects of peptidomimetics for discovery of novel protein scaffolds and therapeutics. The new method has several advantages over bench-scale formats, including reduced reagent and sample consumption, automated handling of liquids and solids, and straightforward parallel-scale synthesis. The results suggest that there is great potential for digital microfluidics for fast and automated combinatorial synthesis of libraries of compounds.

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CONTACT

M.J. Jebrail, tel; +1 925-294-3134; mjebrai@sandia.gov