Parallel combinatorial chemical synthesis using single-layer poly(dimethylsiloxane) microfluidic devices

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Improving methods for high-throughput combinatorial chemistry has emerged as a major area of research because of the importance of rapidly synthesizing large numbers of chemical compounds for drug discovery and other applications. In this investigation, a novel microfluidic chip for performing parallel combinatorial chemical synthesis was developed. Unlike past microfluidic systems designed for parallel combinatorial chemistry, the chip is a single-layer device made of poly-(dimethylsiloxane) that is extremely easy and inexpensive to fabricate. Using the chip, a 2×2 combinatorial series of amide-formation reactions was performed. The results of this combinatorial synthesis indicate that the new device is an effective platform for running parallel organic syntheses at significantly higher throughput than with past methodologies. Additionally, a design algorithm for scaling up the 2×2 combinatorial synthesis chip to address more complex cases was developed. © *2009 American Institute of Physics.* [DOI: 10.1063/1.3230501]

I. INTRODUCTION

Combinatorial chemistry has attracted considerable attention as a means of rapidly producing large numbers of novel compounds with potentially useful biological, chemical, or medicinal properties and has become a ubiquitous procedure in the pharmaceutical industry for generating potential drug candidates.[1] Despite the prevalence of solid-phase methodologies in combinatorial chemistry, a number of advantages of solution-phase combinatorial syntheses have been noted, and solution-phase combinatorial synthesis has developed into a robust field of research.[2] The application of microfluidics to solution-phase combinatorial chemistry has recently attracted considerable attention.[3] Capitalizing on the complementary advantages of solution-phase combinatorial chemistry and microfluidic reactors, several recent studies have reported advances in microfluidic combinatorial chemical synthesis. For example, past achievements include the combinatorial preparation of six 2-aminothiazoles using a Hantzsch synthesis in a heated glass microreactor and the synthesis of a 7×3 library of pyrazoles using a Knorr reaction of 1,3-dicarbonyl compounds with hydrazines.[4] In each of these studies, the reactions were run in a sequential (time-encoded) fashion by performing the component reactions sequentially in a single glass microfluidic channel. Although effective, such a procedure does not allow for synthesizing all of the compounds simultaneously, reducing the throughput of the combinatorial synthesis. Specifically, using a sequential approach to run an $m \times n$ combinatorial synthesis reduces throughput by a factor of mn relative to a parallel methodology. Sequential syntheses using digital microfluidic approaches also suffer from a similar reduction in throughput.[5] Additionally, the repeated use of the same reaction channel to perform multiple syntheses increases the likelihood of cross contamination.[6]

In light of these limitations, a handful of studies have investigated developing microfluidic devices for parallel combinatorial chemical synthesis.[7] In such devices, the constituent reactions

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FIG. 1. Schematic of the chip developed for running a parallel 2×2 combinatorial series of reactions. For a combinatorial synthesis with reactants A1 and A2 in the first library and B1 and B2 in the second library, products A1B1, A2B1, A2B2, and A1B2 are formed. Reactants flow from the inflow ports into the reaction channels via the series of connected y channels; the length of the reaction channels is intended to allow adequate time for diffusive mixing between reactants.

are run simultaneously in separate reaction channels, improving throughput as compared to sequential methods. Parallel combinatorial synthesis of organic compounds has been demonstrated using multiple microreactors on different microfluidic chips;[8] however, such an approach sacrifices the advantages and simplicity of having all reactions integrated onto a single device. Only one major study has been published on parallel microfluidic combinatorial chemistry in which the reactions are run using a single microfluidic device: Kikutani *et al.* recently reported the use of a multilayer glass microfluidic chip to perform a 2×2 combinatorial series of phase-transfer amide-formation reactions.[7] The design they developed for the 2×2 synthesis required that the reaction channels cross at multiple locations, necessitating the use of a multilayer glass microfluidic chip. Their device required only four chemical inputs (one for each of the reactants involved in the 2×2 synthesis) and was demonstrated to be an effective platform for genuinely parallel combinatorial chemical synthesis. However, the multilayer microfluidic chip developed for the investigation was significantly more difficult and expensive to fabricate than a conventional single-layer device, and, due to fabrication complexity, it has been argued that it would be difficult to scale up the design for performing larger combinatorial syntheses.[6]

This investigation addresses limitations to microfluidic combinatorial chemistry by developing and testing a single-layer microfluidic chip for performing parallel combinatorial chemical synthesis. In designing a microfluidic device for parallel combinatorial synthesis, it is possible to disentangle all channels if several of the reactants are inputted at multiple locations, allowing for the development of single-layer microfluidic devices. Using this principle as a basis for design, single-layer microfluidic chips were developed for performing parallel combinatorial syntheses. To demonstrate the effectiveness of the design, a 2×2 combinatorial series of amide-formation reactions was run using one of the chips.

II. EXPERIMENTAL

A. Design of 2×2 parallel synthesis chip

Figure 1 shows an actual-sized schematic of the chip that was developed for performing 2×2 parallel combinatorial chemical syntheses. The key feature of the design is that the chip is a single-layer microfluidic device that is fabricated out of poly(dimethylsiloxane) (PDMS) using soft lithography. The inputs, reaction channels, and outputs are all arranged side by side for ease of use. The chip is the same size as a standard 25×75 mm^2 glass slide, and the channels are approximately 250 μm wide and 50 μm deep. Each inflow channel is 0.5 cm long, and the total length of each reaction channel is 17.1 cm.

B. Chip fabrication

The microfluidic chips were fabricated using conventional soft lithography with SU-8 50 photoresist purchased from Microchem (Newton, MA).[9] The devices are composed of PDMS (Sylgard 184; Dow Corning, Midland, MI) bearing a negative relief of the channel pattern bonded

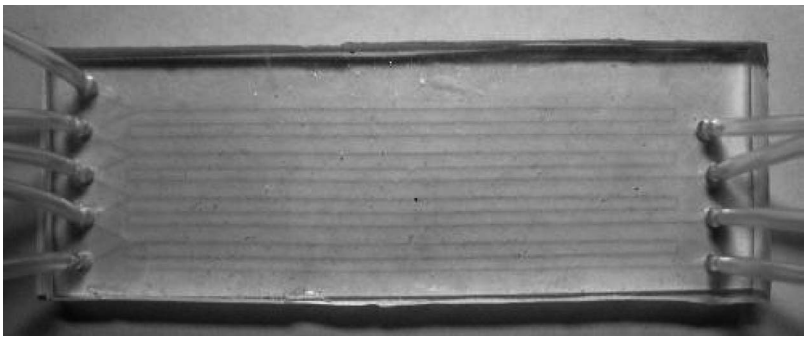


FIG. 2. Photograph of the PDMS microfluidic chip for 2×2 parallel combinatorial synthesis. Tubing is inserted into each of the inflow and outflow ports.

to a glass microscope slide using an oxygen-plasma treatment. The world-to-chip interface consists of flexible small-gauge tubing inserted into holes punched into the PDMS at each inflow and outflow port. A photograph of the completed chip is shown in Fig. 2.

C. Device testing

To demonstrate the efficacy of the chips for doing combinatorial chemistry, a sample 2×2 combinatorial organic synthesis was performed using one of the chips. As is typical in microfluidic organic chemistry experiments, the solution-phase reactants were driven hydrodynamically using a syringe pump.[7,10] Reactants were introduced into the chip by connecting the syringes to the inflow ports using the tubing. The syringe pump drove all of the reactants at an identical rate, ensuring approximately uniform flow and mixing of compounds. The reactant solutions were driven at a volumetric flow rate $Q=0.06$ ml/min, corresponding to an approximate fluid velocity $v=0.08$ m/s. For the experimental conditions in this investigation, the Reynolds number of the chip was calculated to be approximately 14.2 using the equation

$$\mathrm{Re}=\frac{\rho[4(A/P)]v}{\mu},$$

where ρ and μ are the density and dynamic viscosity of the fluid, respectively, and $4(A/P)$ is the hydrodynamic radius (ratio of the cross-sectional area to the wetted perimeter of the channel).[11] The on-chip residence time of the reagents (the ratio of the reaction channel volume to the volumetric flow rate) was approximately 2.1 s.

Products were collected into small glass vials at each of the four outflow ports and were analyzed using conventional gas chromatography–mass spectrometry (GC-MS).

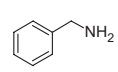
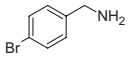
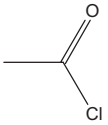
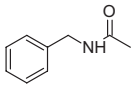
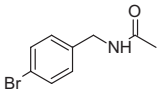
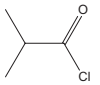
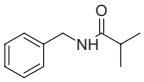
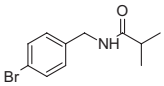
A combinatorial series of Schotten–Baumann reactions to synthesize a library of amides was run to test the chip. In the synthesis, benzylamine and 4-bromobenzylamine were selected as reactants A1 and A2, respectively, and acetyl chloride and isobutyryl chloride were selected as reactants B1 and B2, respectively. Reagent solutions of 0.18M concentration were prepared by dissolving each compound in acetonitrile (ACN); benzylamine and 4-bromobenzylamine were dissolved in the presence of triethylamine.

III. RESULTS AND DISCUSSION

A. 2×2 combinatorial synthesis of amides

Table I shows the structural formula of each of the reactants and products formed in the combinatorial synthesis. Table II lists the mass (m/z) of each of the compounds obtained from the mass spectra and the outflow solution purities determined by comparing peak areas on the gas chromatograms (see Fig. 3). For each solution, the product purity was defined as 1 minus the ratio of the average integral of the impurity peaks to the integral of the product peak. Each uncertainty

TABLE I. Table of the reactants and products comprising the 2×2 combinatorial series of Schotten–Baumann reactions that was run to test the performance of the chip. The synthesis of each of the four amides (products A1B1, A2B1, A1B2, and A2B2) occurred on chip.

	A1	A2
		
B1			
B2			

	A1	A2
B1	A1B1	A2B1
B2	A1B2	A2B2

value is the standard deviation of the average impurity peak integral normalized to the size of the product peak. For the purposes of this analysis, any peak generated by a compound other than the intended product was deemed to be an impurity peak. Sources of the impurity peaks include trace quantities of reactants that did not react, crossover of products from one channel to another due to slight imbalances in flow, and existing impurities in the reagents used.

B. Confirmation of reaction occurrence

An important consideration in microfluidic organic synthesis is to ensure that all reactions occur on chip in the intended reaction channel and not in the off-chip collection vial. Although amide formation from an amine and acid chloride is kinetically extremely rapid,[12] whether the reactions in the combinatorial synthesis occurred on chip is a nontrivial consideration because reagent mixing occurs only by diffusion in the laminar flow regime of the microfluidic chip. The liquid-phase diffusion coefficients of organic molecules such as the reagents used in this investi-

TABLE II. Table listing the mass obtained from the mass spectrum for each product, the purity of each outflow solution, defined as $1-[(A_{\text{impurity}})_{\text{av}}/A_{\text{product}}]$, and the standard deviation of each $(A_{\text{impurity}})_{\text{av}}$ value normalized to the value of A_{product} for that solution.

Product	Mass (m/z)	Purity	SD
A1B1	150.1	0.981	0.0075
A2B1	228.0	0.965	0.047
A1B2	178.1	0.987	0.0091
A2B2	256.0	0.989	0.014

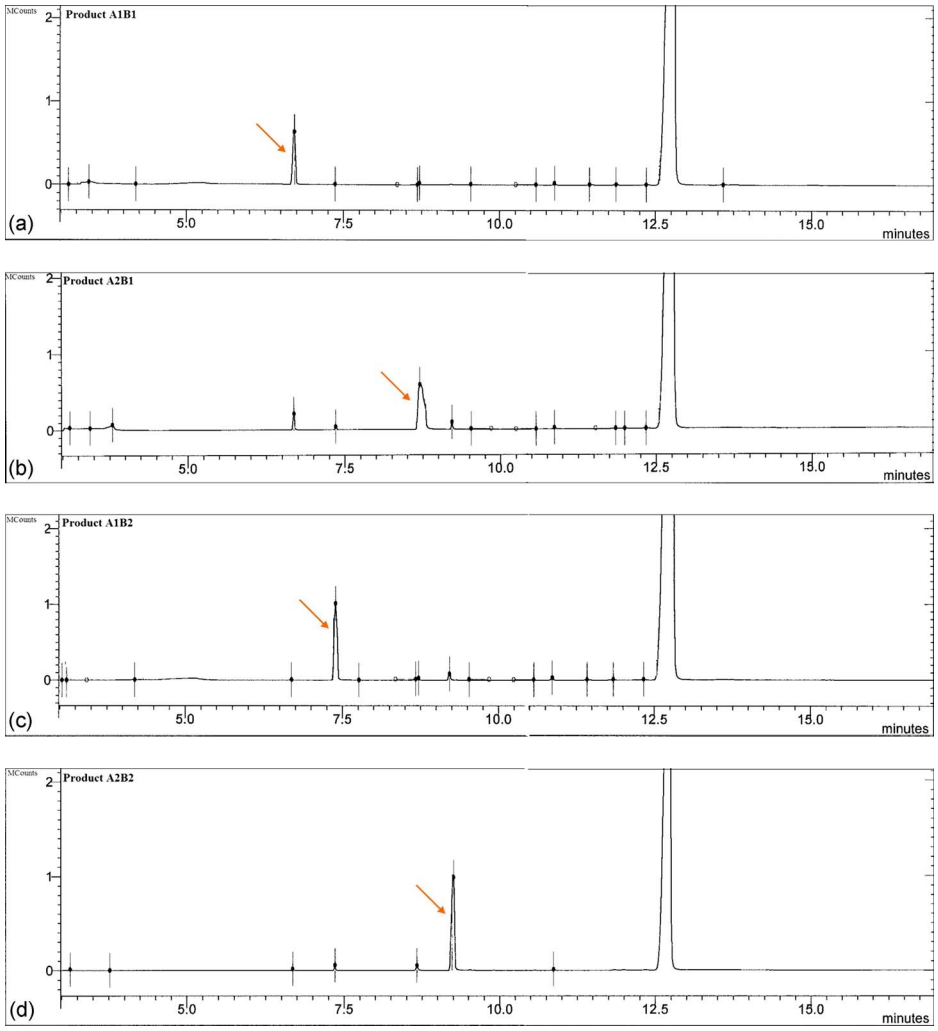


FIG. 3. Gas chromatograms for each of the four outflow solutions that were used to calculate product purity. The peak corresponding to the intended product is denoted with an orange arrow.

gation are typically very small (on the order of 10^{-9} m^2/s) and consequently are difficult to estimate accurately.[13] In turn, this precludes accurate calculation of the time required for the reagents to diffuse across the width of the reaction channel, making it difficult to theoretically confirm on-chip mixing and reaction occurrence.

In light of this, an empirical method for verifying on-chip occurrence of the amide-formation reactions was devised. An additional amide-formation reaction between 0.18*M* solutions of methylbenzylamine and isobutyryl chloride in ACN was run in one of the reaction channels using the same experimental conditions described above for the main combinatorial synthesis. In this synthesis, however, the product solution was flowed directly from the outflow port of the chip into a collection vial containing an excess of benzylamine dissolved in ACN. After completion of the reaction, the entire outflow solution was analyzed using GC-MS. This setup created the potential for a competing amide-formation reaction between benzylamine and isobutyryl chloride to occur off chip if the isobutyryl chloride and methylbenzylamine did not react fully in the channel (see Fig. 4). As such, the absence of any product formed from benzylamine and isobutyryl chloride would provide direct evidence that the on-chip amide synthesis had proceeded as intended. The amide formed from the reaction between methylbenzylamine and isobutyryl chloride (molecular formula $C_{12}H_{17}NO$) has a molecular mass of 191.3, whereas the product of benzylamine and

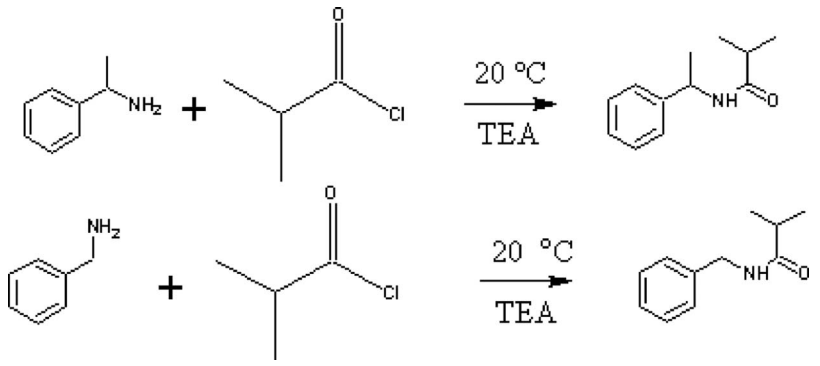


FIG. 4. Equations of the two competing amide-formation reactions. The desired reaction between methylbenzylamine and isobutyryl chloride is depicted on the top, while the undesired reaction between benzylamine and isobutyryl chloride is shown on the bottom. GC-MS performed on the combined outflow solution indicated that the only amide formed was the product of the first reaction and that no reaction between benzylamine and isobutyryl chloride occurred, providing direct evidence that the amide-formation reactions described in the combinatorial synthesis indeed occurred on chip. The molecular formula of the top product is $C_{12}H_{17}NO$ and the formula of the bottom product is $C_{10}H_{14}NO$.

isobutyryl chloride (molecular formula $C_{10}H_{14}NO$) has a mass of 177.2, allowing for easy differentiation between the products using mass spectrometry. From the GC-MS analysis of the outflow solution, only a single amide with a molecular mass of 191.3 was detected, and no compound with a mass of 177.2 was identified, demonstrating, as was expected, that the reaction between benzylamine and isobutyryl chloride did not occur. In a separate experiment, the gas chromatography retention times for the two amides $C_{12}H_{17}NO$ and $C_{10}H_{14}NO$ were both determined to be between 7.0 and 7.5 min. Figure 5 is the relevant section of the gas chromatogram produced from analysis of the outflow solution. It contains only a single peak corresponding to $C_{12}H_{17}NO$, confirming the absence of $C_{10}H_{14}NO$. The results of this test synthesis provide direct evidence for the on-chip occurrence of the series of amide-formation reactions under the experimental conditions employed in this investigation. Furthermore, it would easily be possible to perform kinetically slower reactions, or reactions involving slower-diffusing reactants, using the device simply by decreasing the flow rate to increase on-chip residence time as needed.

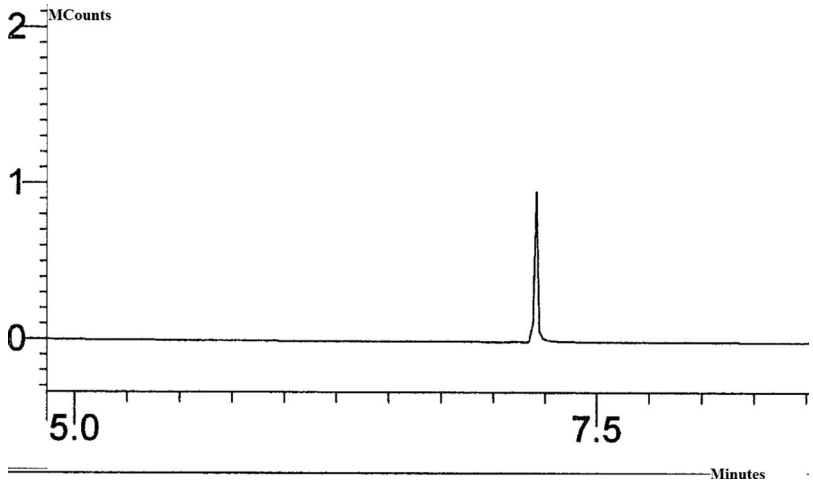


FIG. 5. Gas chromatogram confirming that only one amide-formation reaction (between methylbenzylamine and isobutyryl chloride) occurred.

TABLE III. Table of equations for calculating the number of inputs required for performing an arbitrary combinatorial synthesis. The number of inputs is a function of m, n, and the parity of m and n.

m	n	No. of inputs
Even	Even	$mn+1$
Even	Odd	$mn+m/2$
Odd	Even	$mn+n/2$
Odd	Odd	$mn+(m+n)/2$

C. Scaling up of the design

It should be possible to develop chips for more complicated combinatorial chemical syntheses using the basic principle of design described in this investigation. Combinatorial syntheses involving any number of reactions can be run using single-layer chips similar to the one developed for the 2×2 synthesis by adding inputs, reaction channels, and outputs arranged in the same layout. A series of equations for predicting the number of inputs needed for an $m\times n$ combinatorial series of reactions was derived by modeling the channel patterns as Eulerian graphs and is given in Table III. The number of inputs depends only on the number of reactants in each library and whether the number of compounds is even or odd. In all cases, the total number of reaction channels (and outputs) is 1 less than the number of required inputs. Using this design algorithm, it is possible to quickly develop an exact chip design for running a combinatorial synthesis of any size, enabling the scale up of the chips presented in this investigation to larger and more complicated syntheses. Figure 6 depicts a 3×3 synthesis chip designed and fabricated using this design principle.

FIG. 6. Photograph of the microfluidic chip fabricated for 3×3 parallel combinatorial synthesis. The device was fabricated out of PDMS using the same procedure as for the 2×2 synthesis chip shown in Fig. 2. As predicted by the equations in Table III, the device contains 12 inputs and 11 reaction channels. The chip shown does not have a hole punched at the outflow port of the bottom reaction channel.

IV. CONCLUSIONS

This investigation reports the first parallel combinatorial organic chemical synthesis performed using a single-layer microfluidic device. In contrast to previous microfluidic devices for parallel synthesis, the single-layer chips for performing 2×2 parallel syntheses are simple and inexpensive to fabricate and can be scaled up to run larger series of reactions without a significant increase in cost or fabrication difficulty. The use of a parallel single-layer microfluidic chip increases the throughput of a 2×2 synthesis fourfold compared to a sequential synthesis. This increase in throughput becomes greater for larger syntheses according to the *mn* scaling factor. For instance, it would be possible to run the entire combinatorial series of reactions reported by Garcia-Egido *et al.* in 210 s using one of the scaled up single-layer chips proposed above compared to the 4410 s needed using the sequential chip of Garcia-Egido *et al.*, reducing the total time required to perform the reactions by a factor of 21.[4] The performance of our device compared favorably with that of the chip developed by Kikutani *et al.*;[7] using the chip, we succeeded in producing pure products at a throughput comparable to that achieved using the multilayer glass device. However, our device exhibits the additional major advantage of fabrication simplicity compared to that of Kikutani *et al.*

Minimal cross contamination was observed between products in the test 2×2 synthesis performed on chip. As was suggested previously,[7] this contamination and the variation in degree of contamination for different products were likely due to slightly unequal flow rates for each of the reactant solutions caused by imperfections in the fabrication of the chip and operation of the syringe pumps.

Significantly, no swelling was observed in the PDMS during contact with the reactant solutions, confirming, as previously reported, that ACN is chemically compatible with PDMS.[14] To the best of our knowledge, this investigation constitutes the first report of a combinatorial series of chemical reactions run using a PDMS microfluidic chip. Although PDMS is less chemically robust than glass for organic synthesis applications and is not compatible with a variety of organic solvents, a wide range of organic solvents other than ACN have been demonstrated to be compatible with PDMS, including acetone, *N*-methylpyrrolidone, dimethylformamide, methanol, nitromethane, dimethylsulfoxide, and glycerol.[14] The major advantage to using PDMS instead of glass for the fabrication of combinatorial synthesis chips is that use of PDMS enables a major reduction in fabrication difficulty and cost. Given the range of solvents that are compatible with PDMS, we feel that these advantages in fabrication warrant greater application of PDMS chips to microfluidic organic synthesis. Additionally, there has been considerable progress in the development of polymers more chemically inert than PDMS for use in microfluidic devices, including the fabrication and testing of a microfluidic chip made from a photocurable perfluoropolyether (PFPE) of Rolland *et al.*[15] PFPE devices can also be fabricated using soft lithography, opening the possibility of extending the advantages of our work even to syntheses involving reagents incompatible with PDMS.

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[1] D. Hall, S. Manku, and F. Wang, J. Comb. Chem. **3**, 125 (2001); H. Mario Geysen, F. Schoenen, D. Wagner, and R. Wagner, Nat. Rev. Drug Discovery **2**, 222 (2003); S. Cheng, D. Comer, J. Williams, P. Meyers, and D. Boger, J. Am. Chem. Soc. **118**, 2567 (1996); D. Boger, C. Tarby, P. Meyers, and L. Caporale, *ibid.* **118**, 2109 (1996); C. Selway and N. Terrett, Bioorg. Med. Chem. **4**, 489 (1996); D. Gravert and K. Janda, Bioorg. Med. Chem. **97**, 645 (1997).
[2] H. An and P. Cook, Chem. Rev. (Washington, D.C.) **100**, 3311 (2000); M. Mitchell, V. Spikmans, A. Manz, and A. deMello, J. Chem. Soc., Perkin Trans. 1 2001, 514.
[3] P. Watts and S. Haswell, Curr. Opin. Chem. Biol. **7**, 380 (2003).
[4] E. Garcia-Egido, S. Wong, and B. Warrington, Lab Chip **2**, 31 (2002); E. Garcia-Egido, V. Spikmans, S. Wong, and B. Warrington, *ibid.* **3**, 73 (2003).
[5] T. Hatakeyama, D. Chen, and R. Ismagilov, J. Am. Chem. Soc. **128**, 2518 (2006).

[6] A. deMello, Nature (London) **442**, 394 (2006).
[7] Y. Kikutani, M. Ueno, H. Hisamoto, M. Tokeshi, and T. Kitamori, QSAR Comb. Sci. **24**, 742 (2005); Y. Kikutani, T. Horiuchi, K. Uchiyama, H. Hisamoto, M. Tokeshi, and T. Kitamori, Lab Chip **2**, 188 (2002).
[8] H. Sahoo, J. Kralj, and K. Jensen, Angew. Chem., Int. Ed. **46**, 5704 (2007).
[9] Y. Xia and G. Whitesides, Annu. Rev. Mater. Sci. **28**, 153 (1998).
[10] M. Fernandez-Suarez, S. Wong, and B. Warrington, Lab Chip **2**, 170 (2002).
[11] A. Kamholz, B. Weigl, B. Finlayson, and P. Yager, Anal. Chem. **71**, 5340 (1999).
[12] N. Sonntag, Chem. Rev. (Washington, D.C.) **52**, 237 (1953).
[13] R. Brodkey and H. Hershey, *Transport Phenomena: A Unified Approach* (Brodkey, Columbus, OH, 2003), Vol. 2, Chap. 14, p. 743.
[14] J. Lee, C. Park, and G. Whitesides, Anal. Chem. **75**, 6544 (2003).
[15] J. Rolland, R. Van Dam, D. Schorzman, S. Quake, and J. DeSimone, J. Am. Chem. Soc. **126**, 2322 (2004).