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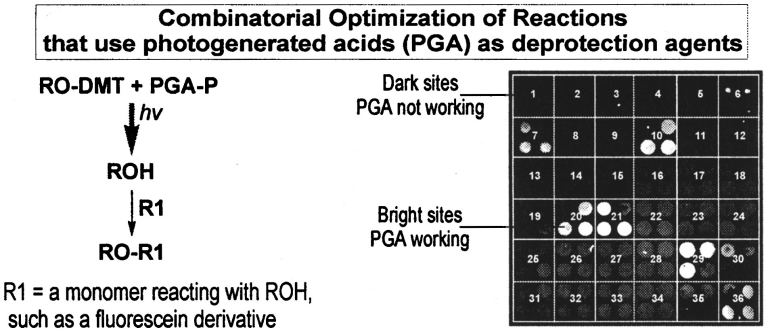

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Digital Light-Directed Synthesis. A Microarray Platform That Permits Rapid Reaction Optimization on a Combinatorial Basis

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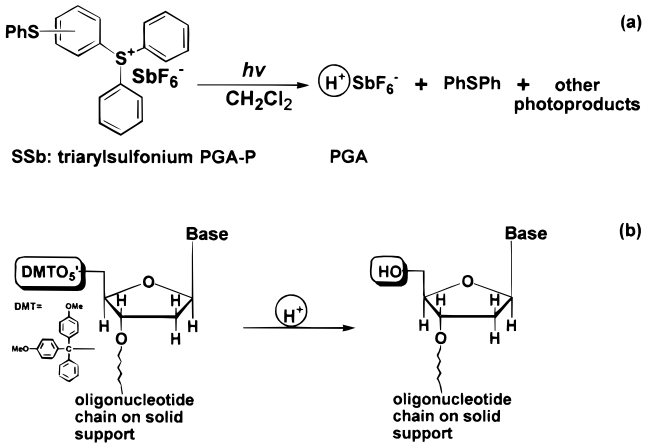
Solution reactions using photogenerated reagents (Gao, X.; Yu, P.; LeProust, E.; Sonigo, L.; Pellois, J. P.; Zhang, H. *J. Am. Chem. Soc.* **1998**, *120*, 12698) are a potentially powerful means for combinatorial parallel synthesis of addressable molecular microarrays. In this report, we demonstrate that this chemistry permits combinatorial screening of reaction conditions on a microarray platform. Using this method of optimization and our reaction apparatus, efficient photogenerated acids and reaction conditions suitable for removal of the acid labile protection group on 5′-O of nucleotides are identified in a short period of time. The chemistry platform demonstrated opens new avenues for rapid, simultaneous investigation of multiple reactions using different reagents and reaction parameters directly on a solid support (e.g., a glass plate). The combinatorial screening method described may be extended to include general organic reactions employing photogenerated and conventional reagents as well as a microarray reaction device. This should be especially valuable for efficient synthesis of addressable organic compound libraries.

Introduction

The progress in combinatorial synthesis has brought about unprecedented opportunities for efficiently creating libraries of diverse molecules.[1] In these reactions, a scaffold chemical moiety containing at least one reactive site is reacted with a number of different compounds to yield a library of derivatives differing in substituent groups. Since hundreds to thousands of diverse compounds are generated in combinatorial syntheses, it is desirable to establish high yield reactions so that purification steps may be reduced or eliminated and data interpretation may be simplified. Therefore, it is essential to optimize reaction conditions with respect to reagents, solvents, temperature, reaction time, and so forth. In practice, this has been the most effort-consuming step in designing a combinatorial synthesis. Usually various reaction conditions are individually tested, and thus only a limited number of conditions can be examined within a reasonable time frame. In this report, using the removal of acid labile protecting groups as an example, we describe the application of a novel solution photochemistry toward combinatorial screening of reaction conditions that permits in situ rapid, simultaneous investigation of multiple reactions using different reagents and reaction parameters. The screening reactions described demonstrate the power of a digital light-controlled microarray platform[2] (a microarray reactor, a micromirror array image projector, a controller computer, and an automated synthesizer; Scheme S1, Supporting Information) in speeding up combinatorial syntheses on solid supports.

Scheme 1. Illustration of PGA Deprotection of the Acid Labile Protecting Group



The reported work was a result of our development of digital light-directed synthesis that uses photogenerated reagents to effect conventional chemistry (Scheme 1).[3] To prove the concept, we demonstrated the applicability of the chemistry for solution deprotection of 5′-O-DMT (DMT: 4,4′-dimethoxytrityl) using photogenerated acids (PGAs for cleavage of the ether C−O bond, Scheme 1) and the subsequent synthesis of oligonucleotides. These reactions were performed on bulk solid support (controlled porous

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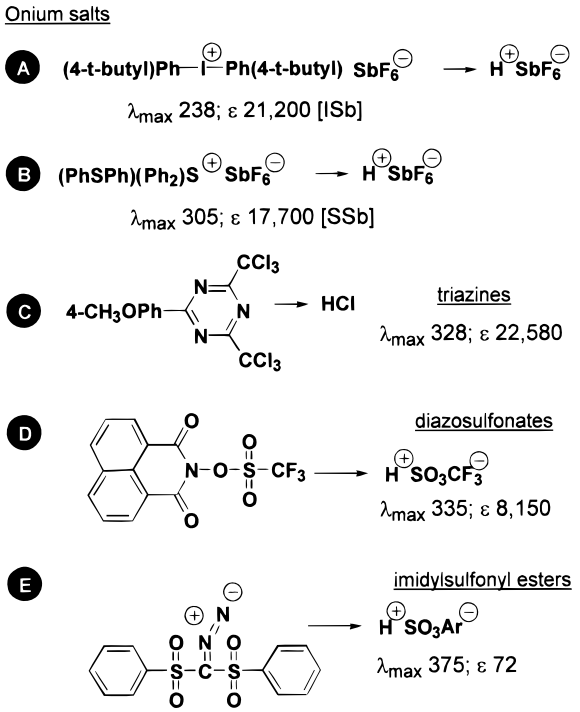
glass, CPG) in a single solution reaction vessel. To implement the chemistry on a microarray platform, it is necessary for us to identify the PGA precursors (PGA-Ps) and the solution reaction conditions that are efficient for the removal of the acid labile protecting groups as well as compatible with the subsequent synthesis of oligonucleotide arrays. Although a large number of PGA-Ps and ample examples of their applications can be found in the literature,[4] these works are mainly based on the reactions using chemical amplification (i.e., autocatalyzed reactions) carried out in polymeric solid phase (i.e., photoresist processes). Clearly, there are fundamental differences between the reactions occurring in a polymer phase and those occurring in a conventional solution phase. For instance, diffusion is much slower in polymer media than in solution, and the reactivities of the reagents and the reaction intermediates buried in polymer materials versus those mediated by a solution would be very different. Furthermore, the micromirror projector used for reactions requires a 400 nm or longer wavelength; in contrast, the photoresist process used in microelectronics involves short wavelength illumination. We soon found that even the reaction conditions that produced complete deprotection in bulk solution were not necessarily directly applicable to solution deprotection on a glass plate. Therefore, a multiple of reaction parameters need to be optimized. It was necessary for us to perform, on glass surface, a multidimensional search for the best combination of the various reaction conditions, such as stoichiometric ratio of PGA-P and/or coreagents, solvents, and cosolvents, light irradiation wavelength, illumination intensity and time, reaction time, pre-/postreaction treatment, and so forth.

Materials and Methods

The microarray reaction apparatus used consists of a microarray reactor (Scheme S1[2]), a computer controller, a light source (Hg or Hg/Xe lamp, 200 W, Oriel Instruments), a micromirror chip containing 480 × 640 pieces of mirrors of 16 × 16 μm (Texas Instruments), and a DNA synthesizer (Expedite 8909, PE Biosystems). A program developed in-house provided digital patterns of light irradiation, and conventional protocols implemented in the DNA synthesizer were used for the synthesis of oligonucleotides. The microflow patterns of the microarray reactor were monitored in real time by taking imaging through a microscopic video camera and viewing through a monitor (Sony).

Glass Plate Derivatization. Microarray glass plates containing nonwetting film patterns[5] of $m \times m$ (m = 12, 24, and 48) reaction sites in circular or square shapes were fabricated using standard photolithography techniques.[6] Polyimide photoresist (Clariant) film coated on the surface of glass plates was patterned to protect microreaction wells on the surface and then reacted with a solution of (heptadecafluoro-1,1,2,2-tetrahydrodecyl)triethoxysilane (Gelest) in cyclohexane (20 mM, 20 mL) overnight at room temperature with gentle shaking. The glass plate was then rinsed with cyclohexane (ca. 20 mL) and cured at 100 °C for 1 h under ultradry N_2. The polyimide layer was removed by successive washing with acetone and 2-propanol. The patterned area of microarrays have a dimension of 1.5 × 1.5 cm^2, and the

Scheme 2. Examples of PGA[a]



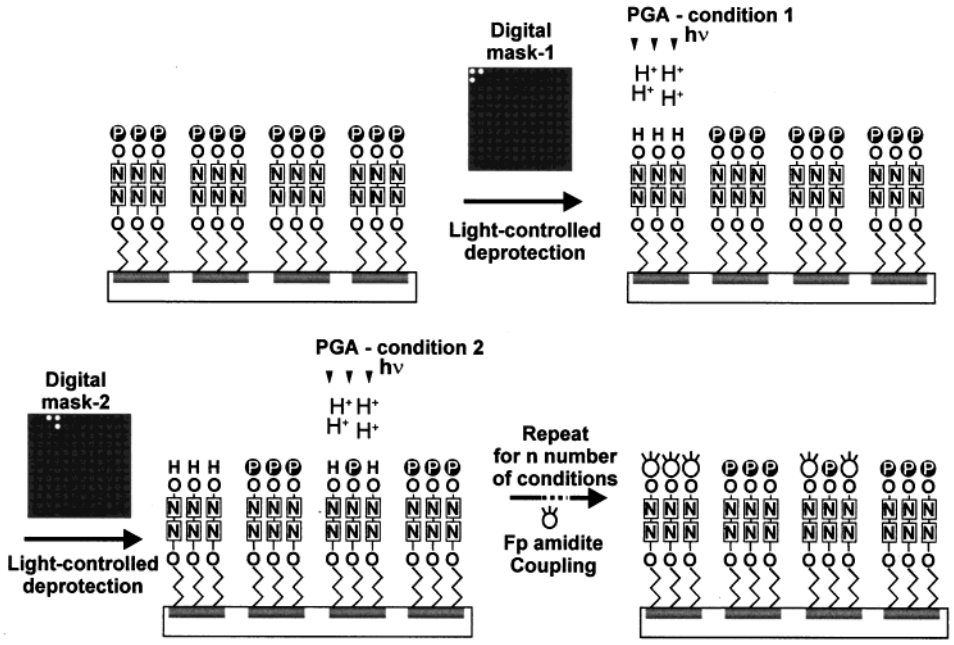
[a] UV parameters from the catalog of Madori Kagaku Ltd.

12 × 12, 24 × 24, and 48 × 48 microreaction wells have corresponding features of 1200, 900, and 300 μm. Before derivatization with linker molecules within microreaction wells, the glass plate was treated with hot piranha solution (concentrated H_2SO_4:H_2O_2, 50:50 v/v) for 15 min[7] and then thoroughly rinsed with H_2O and EtOH and dried. To the cleaned glass plate was added *N*-(3-triethoxysilylpropyl)-4-hydroxybutyramide (Gelest or Lancasteer) (1% v/v in 95% EtOH). The reaction was left at room temperature for at least 1 h with gentle shaking.[8] Upon completion of the reaction, the glass plate was rinsed with 95% EtOH and cured at 100 °C for 1 h under ultradry N_2. The derivatized plates were stored in a clean and dry container.

Combinatorial Screening Reactions Using the Micro array Reaction Apparatus. PGA-P compounds (Scheme 2) included onium salts (Secant) (compounds A and B, Scheme 2), a triazine (Midori Kagaku) (compound C, Scheme 2), an imidylsulfonyl ester (Midori Kagaku) (compound D, Scheme 2), and a diazosulfonate (Secant) (compound E, Scheme 2). Thioxanthenone (Aldrich) and propylene carbonate (Aldrich) were used as sensitizer and stabilizer (or hydrogen donor), respectively. The PGA-P solutions were prepared in a dark room to have final concentrations ranging from 1.0 to 65.0 mM. These solutions are stable for at least 14 days when stored in the dark at room temperature. The molar ratio of sensitizer/stabilizer to PGA-P used in the testing experiments varied from 0:1 to 100:1. Specific ratios of the reagents used will be given below.

The derivatized microarray plate was placed in a microarray reactor that was mounted to the synthesizer to allow automated reactions. The microreaction wells were aligned, with the assistance of a video camera, allowing the digital pattern of light to be projected onto the glass plate from the micromirror chip. The light source was turned on during the

Scheme 3. Combinatorial Optimization of Reaction Conditions



deprotection step in synthesis cycles so that the light was reflected from the micromirror to the microarray plate. The reactions began with uniform growth of 5′-DMT-O-TT (T = thymidine) on surface HO-linkers within all reaction wells, using standard phosphoramidites[9] (Glen Research) and TCA (trichloroacetic acid, 2% in CH_2Cl_2) as the deprotecting reagent. The following steps represent combinatorial screening of reaction conditions for deprotection of the 5′-O-DMT group using PGA in solution (Scheme 3). The microarray reactor was flushed with CH_3CN (3×) and purged with N_2 for 1 min, and the first PGA-P solution (condition #1, 5 mM compound A in CH_2Cl_2, Scheme 2), was manually injected into the reactor using a syringe (this was the only manual step in the entire synthesis process). The PGA-P solution was drained from the reactor to allow droplets to form at the reaction sites confined by the nonwetting film patterned on the glass plate. Thus, each droplet contained the first PGA-P solution. The glass plate was irradiated with light (10 mW/cm^2, 405 nm) projected from the micromirror chip, according to the predetermined pattern (Scheme 3, Figure 1, four wells for each reaction condition, three wells were irradiated and one well was not irradiated). The irradiation time was 1 min. After the light was turned off, the reaction was allowed to continue for another 0.5 min. The reactor was then flushed with pyridine/CH_3CN (1×) and CH_3CN (2×). The glass plate was then again filled with the PGA-P solution of the second condition as shown in Scheme 3 (condition #2, 30 mM compound A in CH_2Cl_2, Scheme 2), and the light irradiation and rinsing steps were repeated as described. The cycles of PGA deprotection were repeated *n* times (*n* is the number of different deprotection reaction conditions). The glass plate was then treated with Fp (0.1 M, Fp:Tp was 1:10 mol/mol) in CH_3CN to couple the fluorophore to the 5′-OH groups released by the PGA deprotection (Scheme 4). Following the coupling reaction, oxidation and rinsing were carried out as in regular DNA synthesis. In the set of experiments shown in Figure 1, the

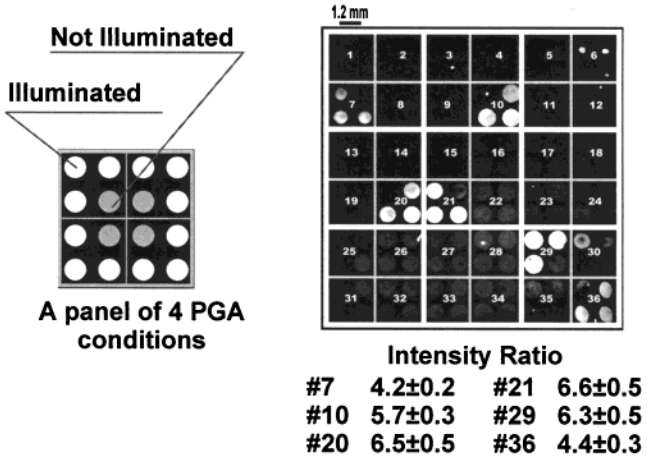
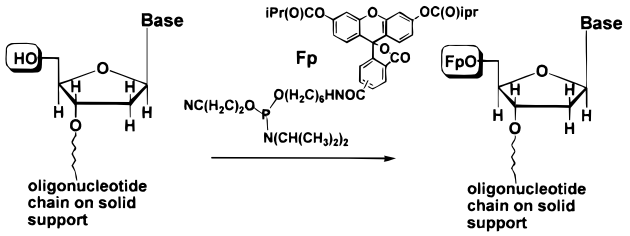


Figure 1. Fluorescence image of the combinatorial screening of 36 PGA-P solution reaction conditions for deprotection of 5′-O-DMT of the T nucleotide. The reaction wells with higher fluorescence intensities indicate more suitable deprotection reaction conditions.

Scheme 4. Coupling Reaction of Fluorescein Phosphoramidite



PGA-P compounds from the four families of PGA-P (Scheme 2) with concentrations varying from 1.0 to 65.0 mM were examined. The ratio of photosensitizer (an energy transfer compound which itself may not undergo chemical conversion in the reaction) thioxanthenone:PGA-P was 0, 1, 2, 10, or 100:1 for the same PGA-P. The ratio of stabilizer (a hydrogen donor) to PGA-P was 1 and 100:1 for the same PGA-P and photosensitizer. Other reaction parameters varied were light

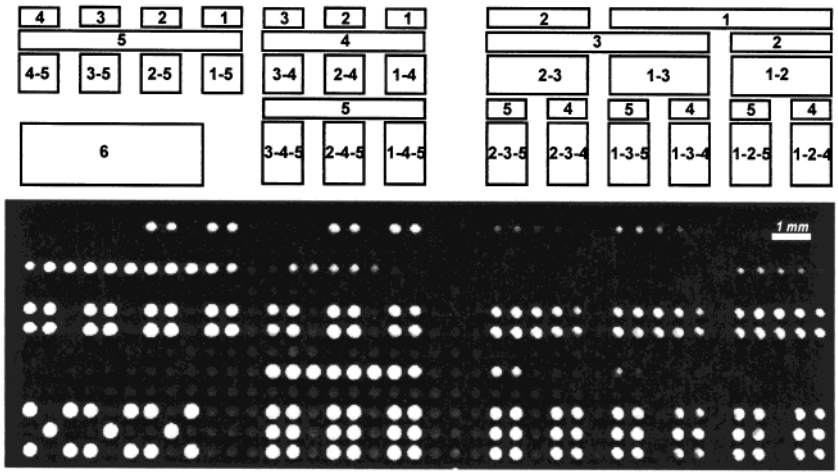


Figure 2. Fluorescence image of the combinatorial screening of PGA-P solution reaction conditions for the deprotection of 5′-O-DMT. The combinatorial reaction pattern is explained in the boxes above the image. The first deprotection cycle involved reaction wells labeled with 1; the second deprotection cycle involved reaction wells labeled with 2, etc. In cycles 1−5 the deprotection reaction was performed more than once (separate numbers in the box indicated number of deprotection reaction performed) and cycle 6 was a control, which deprotected DMT efficiently.

irradiation time (0.5, 1, 1.5, and 2 min) and the sum of reaction times for irradiation and incubation (total less than 3 min). Light wavelength (365 and 405 nm) and intensity (6−10 mW/cm^2) were also systematically investigated.

The combinatorial screening was carried out in an iterative manner. The optimal reaction conditions identified from initial screenings provided leads for the subsequent focused screening to examine reaction parameters that were more relevant to the outcome of DMT deprotection. One example is illustrated in Figure 2 where reaction wells were subjected one, two, or three times to PGA [compound A (60 mM) (Scheme 2) and thioxanthenone (2 equiv), 1 min irradiation each time] treatments. In the reaction shown in Figure 2, conditions #1−5 used the same PGA-P solution and were found to deprotect DMT quantitatively after at least two deprotection cycles or equivalent deprotection time used. Condition #6 was a different PGA-P application condition and achieved the same result in one cycle only. The subsequent screening reactions produced consistent results, such as those shown in Figure 1. The completion of PGA detrytilation was examined by a second deprotection using TCA following Fp coupling and capping with the acetyl group. This will expose those which were not deprotected by the previous treatment of PGA. A second Fp coupling was then performed. Detritylation was complete if these reactions did not result in an increase of fluorescent intensity.

Imaging of the Combinatorial Microarray Plate. The reacted glass plate was treated with ethylenediamine (50% in dry EtOH, 2 h), followed by thorough washing with EtOH and acetone. The dried plate was placed under a cooled CCD camera (Apogee Instruments). The fluorophore excitation and detection were at 494 and 525 nm, respectively, using the same light source as for photodeprotection reactions (Zhou, unpublished results). The fluorescent image of the plate was taken, processed, and analyzed using the Image Pro program (Media Imagenics). The intensities were measured, and the average values of individual reaction wells were calculated. The average ratios and deviations of the intensity of the irradiated wells versus the reference dark wells were derived (Figure 1).

Results and Discussion

General Reaction Scheme. Our experiments employed glass plates fabricated to contain microarrays of reaction wells (e.g., 144, 576, or 2304 wells in an area of 1.5 × 1.5 cm^2). The microwells were defined by a monomolecular layer of nonwetting film[5] covalently bonded on the glass plate. The surface of the microwells was derivatized with 5′-DMT-O-T_2-O- sequences through immobilized silane linkers.[10] A solution containing PGA-P and possibly other co-reagents was delivered to the microarray plate in the microarray reactor using the DNA synthesizer (Scheme S1), and the solution was subsequently drained. This caused the formation of isolated droplets within each microwell due to the nonwetting surface tension. A digital illumination pattern (Scheme 3) generated using a computer controlled micromirror projector (Digital Light Processing (DLP) projector) was projected onto the reaction wells on the glass plate. The illumination patterns conformed to preselected reaction sites as shown in Figure 1. In this experiment, 36 illumination patterns were used to test 36 sets of reaction conditions. Each reaction set involved four reaction microwells: three were irradiated during the reaction, while the fourth one was not illuminated and used as a reference (Figure 1). The glass plate was then washed with CH_3CN. Next, PGA-P solution was applied, and the deprotection using different reaction conditions was performed (Scheme 3 and Figure 1). This cycle of deprotection using different reaction conditions was repeated until the designed sets of reaction conditions were all examined.

PGA Selection. In the experiment shown in Figure 1, the combinatorial screening procedures allowed simultaneous investigation of 36 reaction conditions. The PGA-P compounds used are given in Scheme 2, including an iodium salt (compound A, Scheme 2), a sulfonium salt (compound B, Scheme 2), a triazine (compound C, Scheme 2), an imidylsulfonyl ester (compound D, Scheme 2), and a

diazosulfonate (compound E, Scheme 2). Compounds A−D have maximum absorption far below the 400 nm required by the light projector; therefore, it was necessary to use a sensitizer (thioxanthenone, λ_{max} 400 nm, ϵ 2557), which has a much stronger absorption at 400 nm, to induce the formation of acid from them. We were also interested in the effect of stabilizer, which is, in general, an efficient hydrogen donor. Propylene carbonate is a common component used in PGA systems,[4a] and thus it was used in some of the reactions. In these experiments, the concentrations of the PGA-P compounds were 1, 2, 5, 10, 30, and 60 mM. The ratio of PGA-P to photosensitizer and/or stabilizer were varied. At the nonirradiated reaction sites the 5′-O-DMT group was expected to remain intact. At irradiated reaction sites, effective PGA would remove the 5′-O-DMT group. The removal of the DMT group resulted in formation of 5′-OH, which was assayed by coupling it with the fluorescein phosphoramidite (Fp, Scheme 4) followed by oxidation using I_2. Fp to thymidine phosphoramidite in a 1:10 ratio was used to ensure linear fluorescence response by avoiding the fluorescence quenching effect.[10] The experiment for screening 36 reaction conditions was completed in 20 h including the time for experimental design and the preparation of the PGA-P solutions. The results of the combinatorial screening were imaged using a cooled CCD camera.

Figure 1 displays the fluorescence image obtained from the screening experiment described above. The sites with higher fluorescence intensity correspond to more efficient removal of the DMT groups and thus correlate with more suitable deprotection conditions. The sites of low and null fluorescence intensity correspond to partial or failed 5′-O-DMT deprotection, and thus indicate undesirable deprotection conditions. Figure 1 reveals that among the 36 reaction conditions, six sets are better conditions than others. The fluorescent intensity of the six sets was measured to give the following reading (ratio of the bright versus the dark reference spot): #7, 4.2 ± 0.2; #10, 5.7 ± 0.3; #20, 6.5 ± 0.5; #21, 6.6 ±0.5; #29, 6.3 ± 0.5; and #36, 4.4 ± 0.3. These results and other screening tests suggest that under given light irradiation conditions (10 mW/cm^2 and 405 nm) compound A (Scheme 2) and its analogues/photosensitizer (1−2 equiv) combinations are effective DMT deprotection reaction conditions. Our previous synthesis of oligonucleotides on CPG used triarylsulfonium hexafluoroantimonate in CH_2Cl_2.[3] However, this PGA exhibited nearly no reactivity when applied in the microarray reactions, since the compound has very weak absorptions at wavelengths above 400 nm. The described combinatorial screen reactions allowed us to rapidly zoom in onto only selected few PGA compounds from a quite large number of candidates (vide supra).

Factors Affecting the PGA Deprotection Reaction. On the basis of these results, the selected sets of reaction conditions were further explored using additional reaction parameters, such as reaction time and varied light irradiation conditions (Figure 2, focused screening). Some selected sites were repeatedly treated with PGA conditions, and the deprotection results are shown in fluorescent display (Figure 2). These results show that sufficient contact between PGA and the surface molecules is important for quantitative deprotection of the DMT group, which was achieved by multiple PGA treatments (Figure 2, reaction wells labeled with 4−5, 3−5, etc.). These effective PGA compounds and their identified deprotection conditions were further confirmed in the synthesis of oligonucleotides on glass plates (this laboratory, unpublished results[11]).

Different solvents for PGA deprotection were examined using condition #21 (Material and Methods) except for the sovlent, since it is desirable to have a less volatile solvent that retains the droplet for a longer time than CH_2Cl_2. The solvents used in these comparison studies included CH_2Cl_2, $CHCl_3$, CH_3CCl_3, $(ClH_2C)_2$, and trichlorobenzene. With all the solvents tested, CH_2Cl_2 produced the best results, based on the final Fp assay for the reactions. Additionally, when a mixed solvent, such as propylene carbonate/CH_2Cl_2, was used, an increased amount of the former (from 0.086:1 to 0.667:1) gave lower fluorescent reading. The significant solvent effect was not completely expected and requires further investigation.

The use of the combinatorial screening method permits us to not only identify effective PGA-P reagents but also establish suitable reaction conditions for high yield cleavage of the acid labile DMT. Parallel comparison of the PGA deprotection reaction with that using trichloroacetic acid (TCA, conventionally used for DMT deprotection) indicates equivalent performance of the two systems in the corresponding fluorescence assays.

Summary and Conclusion

This work reports a new chemistry platform based on solution digital light-directed reactions and a microarray reaction device. The method of combinatorial optimization of reactions described above involves the use of photogenerated reagents for selective deprotection at the reaction sites that are designated to undergo next reaction. This screening method should be especially valuable for optimized synthesis of addressable organic compound libraries. A general strategy for synthesis of a library of molecules would involve protection of the reactive group in one of the reactants (immobilized or in solution), thus allowing the protected compounds at selectively irradiated reaction sites to be deprotected and able to react with incoming compounds. Repeating the deprotection and the reaction steps within different reaction wells should allow diverse molecules to be synthesized. These reactions are simple in operation, since they are light-gated rather than controlled mechanically, and easy to adopt, since there is no need to prepare photolabile protecting group protected starting materials as required by the existing method of light-directed synthesis of oligonucleotides and peptides.[12]

There is a need to explore an expanded list of PGA and photogenerated reagents in general to be used in solution for diverse chemical reactions. A large number of PGA-Ps are available for the removal of the acid labile protecting groups.[4] Photogenerated bases (PGB) have been reported but found limited applications in the removal of the base labile protecting groups.[13] Other types of photogenerated reagents, including PGB, remain to be developed. In retrospect, there are numerous examples of compounds containing photolabile

protecting groups. These compounds are potential photogenerated reagents that can assist reactions upon light activation. Efforts are underway in this laboratory to demonstrate parallel syntheses of organic compound libraries on a digital light-controlled microarray platform as described in this report.

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Supporting Information Available. Schematic of the microarray platform used in this study. This material is available free of charge via the Internet at http://pubs.acs.org.

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