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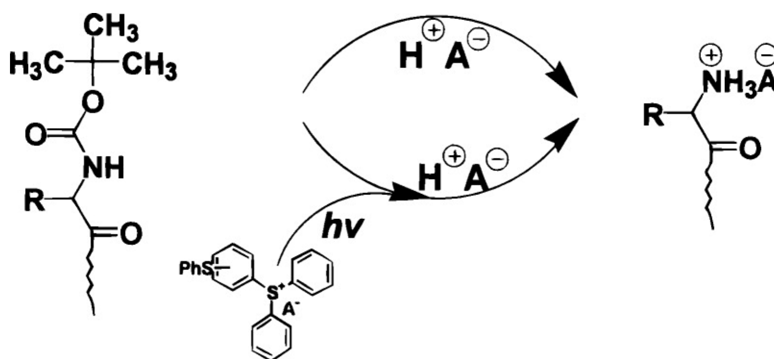

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Peptide Synthesis Based on *t*-Boc Chemistry and Solution Photogenerated Acids

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Solution reactions using photogenerated reagents (PGRs) (Gao, X.; Yu, P. Y.; Leproust, E.; Sonigo, L.; Pellois, J. P.; Zhang, H. *J. Am. Chem. Soc*. **1998**, *120*, 12698) are developed for parallel synthesis of addressable, combinatorial molecular microarrays. To advance the PGR chemistry for general chemical conversions, light-controlled synthesis of peptides, which employs photogenerated acids (PGAs) and/or in combination with photosensitizers for deprotection of N-*t*-Boc group, is demonstrated. These reactions were performed on resin and glass plates and conveniently monitored by HPLC analysis (reactions on resin) and fluorescence emission after coupling the deprotected NH_2 group with 4(5)-carboxyfluorescein. These results demonstrate the potential of the PGA chemistry for parallel synthesis of addressable peptide libraries on a microarray platform.

Introduction

Solid-phase synthesis[1] represented a revolutionary advancement in the history of organic synthesis. Three decades later, the second advancement of equal importance, i.e., parallel synthesis of molecular libraries on solid supports,[2] is increasingly penetrating the fields of chemical synthesis. Of the various libraries of organic/bioorganic compounds, those of oligonucleotides and peptides are especially useful due to their essential role in every form of life. Peptide libraries, for instance, have been successfully used in epitope mapping for receptors, antibodies, and other target proteins.[3] For the generation of structure-tractable, large peptide libraries using chemical means, two major methods, which are the split-and-mix (SAM)[4] and the light-directed syntheses,[2e,2f] are available, with the former widely adopted in library synthesis. However, both methods suffer from limitations. The SAM approach relies on special ways of tagging/coding the beads to register structural information on a specific bead. The photolithography method requires photolabile protection groups in amino acid monomers. This process, in theory, has the advantage of generating addressable arrays. The process, however, is difficult to replicate in a regular laboratory setting and has only limited use. This is because the chemistry used inherently is not as clean as conventional peptide synthesis and the process is highly expensive and inflexible. There still are great demands for new synthetic strategies, especially for better methods of parallel synthesis of a large number of peptides.

We rationalized that the photolithographic synthesis[2e,2f] can become a laboratory routine if the selective light activation step can be applied without altering the conditions of conventional synthesis in a multiple reaction setting. We have since demonstrated that this can be achieved by using solution photogenerated reagents, on a microarray platform.[5] This method of combinatorial synthesis has distinct advantages, as it is simple, flexible, and easy to adopt in a regular research environment. Our method would be especially suitable for the synthesis of addressable peptide arrays as it does not require as many as 20 photolabile group protected amino acid monomers nor a large number of photomasks (maximum number of masks $= 20 \times$ residue number in the sequence), which are necessary for the existing photolithographic synthesis.[2e] In the present work, we explore the synthesis of peptides using our novel solution photochemistry and investigate the photogenerated acids (PGAs) suitable for these reactions. The results of this work form the foundation for automated synthesis of addressable peptide microarrays.

Materials and Methods

The *tert*-butyloxycarbonyl (Boc)-amino acids, the HMBA-AM resin, and hydroxybenzotriazole (HOBt) were purchased from Novabiochem (La Jolla, CA); 1,3-diisopropylcarbodiimide (DIC) and thioxanthenone were from Aldrich (Milwaukee, WI); 4-(dimethylamino)pyridine (DMAP) was from Sigma (St. Louis, MO); 3-aminopropyltriethoxysilane from Lancaster (Gainesville, FL); and trifluoroacetic acid (TFA) 99.97% was from Chem-Impex International (Wood Dale, IL). Photogenerated acid precursors (PGA-Ps) (Scheme 2), 50% triarylsulfonium hexafluoroantimonate in propylene carbonate (SSb), and diaryl iodium hexafluoroantimonate (ISb) were generous gifts from Secant Chemicals Inc. (MA) and Midori Kagaku Co., Ltd. (Japan), respectively. Most solvents and reagents were used without further purification,

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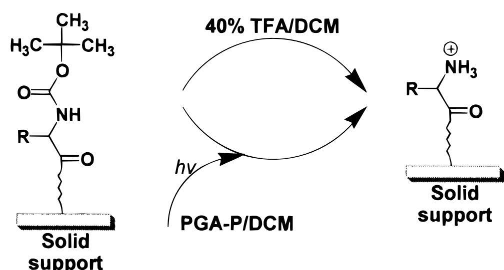
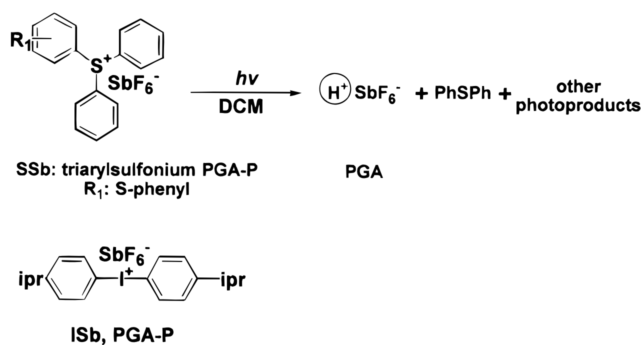
Scheme 1



Scheme 2. Example of the Photoreaction of PGA



except for dimethyl formamide (DMF), which was distilled before use. Peptide synthesis on resin was carried out at room temperature in a vessel for manual solid-phase peptide synthesis. Light illumination was performed using either a UVP lamp (UVGL-58, 366 nm, 720 μW) or a collimated light source equipped with a 200 W Hg−Xe lamp (model 66033, Oriel Instruments). Selective irradiation at a wavelength longer than 400 nm was carried out using the light source with the Hg line filters (10 nm bandwidth, Oriel). Irradiance was measured using a monochromic photometer (model 840-c, Newport). All photoreactions were performed in a dark room, and the photoreagent bottles were shielded from direct light by wrapping with aluminum foil. Deprotection and coupling steps were monitored using ninhydrin test. HPLC analyses of the deprotection and coupling reactions were performed on either an HP 1090 or a Waters M510 dual pump unit equipped with a photodiode array detector (966 PDA). Columns were reverse-phase (RP-C18, Vydac; 8NVC84μ, Waters). Elute solvents were water (A) and acetonitrile (B) both containing 0.1% TFA. Linear gradients of 10−70% of A in B in 25 min with a flow rate of 1.5 mL/min (Vydac 4.6 × 20 column) and 15−80% of A in B in 13 min with a flow rate of 3 mL/min (Waters 10 × 10 column) were used. Semipreparative HPLC was performed on a reverse-phase C_{18} column (Waters' HC18 HA) using a linear gradient of 15−80% of A in B in 60 min. ^{1}H NMR spectra were recorded on a Bruker AMX-II 600 MHz spectrometer.

Derivatization of Boc-Tyr(Bzl)-HMBA-AM Resin. HMBA-AM resin (30 mg, 30 μmol) in DMF/DCM (dicholoromethane) (v/v 1/1, unless otherwise specified, 1 mL) was treated with Boc-Tyr(Bzl)-OH (33.4 mg, 90 μmol, Bzl: benzyl) in the presence of DIC (14 μL, 90 μmol) and DMAP (3.7 mg, 30 μmol) for 3 h. The derivatized resin was washed thoroughly using DMF and DCM, dried under vacuum, and stored in a dry place.

Table 1. Reaction Condition Summary: Deprotection of N-Boc-Tyr(Bzl)-resin[a]

reagent	acid conditions	reaction time (min)	yield (%)[b]
TFA	40% (5.1 M), 1.00 mL, 519 equiv	30	100
SSb (PGA)	5.5% (90 mM), 1 mL, 9 equiv	2	30
		4	70
		8	94
		16	100
SSb (PGA)	2.4% (40 mM), 3 mL, 12 equiv	2	68
		4	94
		8	97
		16	97
SSb (PGA)	1.2% (20 mM), 6 mL, 12 equiv	16	99

[a] Reaction used 10 μmol of amino acid on resin. All deprotection reactions used DCM as the solvent. Light irradiation was at 365 nm, 22 mW. [b] Deprotection yield percentage was obtained from HPLC peak integration.

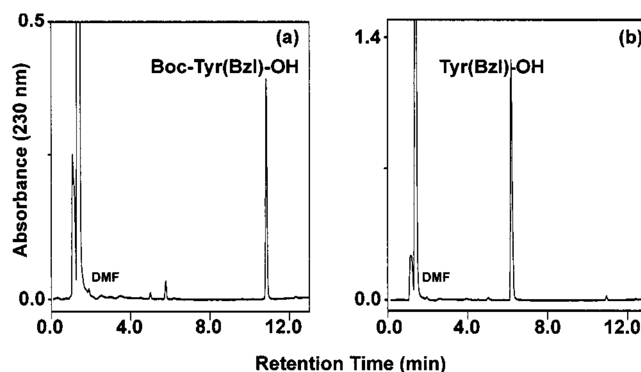


Figure 1. HPLC profiles of (a) Boc-Tyr(Bzl)-OH and (b) Tyr-(Bzl)-OH obtained using solution PGA deprotection.

Deprotection of the Terminal N-Boc Group Using PGA (Scheme 1). To Boc-Tyr(Bzl)-HMBA-AM resin (13.5 mg, 10 μmol) was added SSb (Scheme 2) in DCM (concentrations are given in Table 1). In one reaction set, the solution was left in the dark for at least 30 min. This reaction produced a negative ninhydrin test. In other sets, the reaction solution was UV illuminated (365 nm) for various times (Table 1). The amino acid on resin was then treated with NaOH (1.0 M, 180 μL) in DMF (20 μL) for 1 h as recommended by the vendor for peptide cleavage from the resin. The PGA deprotection product was examined using HPLC. Boc-Tyr(Bzl)-OH and Tyr(Bzl)-OH, obtained from Boc-Tyr(Bzl)-OH treated with 40% TFA in DCM, were used as the reference compounds, confirming identical HPLC retention times and UV absorption profiles. A representative HPLC profile after complete deprotection of the Boc group from Tyr(Bzl)-OH is shown in Figure 1. Deprotection reaction efficiency as a function of deprotection reaction time is summarized in Table 1.

The reaction was also performed as described above but deprotection reagents were PGA-P ISb (λ_{max} 238 nm, ϵ 21,200 M^{-1} cm^{-1}) (Scheme 2, 5 mM in DCM) using 1−2 equiv of the Boc group and photosensitizer (an energy transfer compound which itself may not undergo chemical conversion in the reaction) thioxanthenone (λ_{max} 400 nm, ϵ 2,557 M^{-1} cm^{-1}) in ratios of 1:1 to 1:100 (PGA-P:sensitizer). UV irradiation was at 404.7 nm, 10 mW/cm^2, and the illumination lasted 30 min. After cleavage from resin, the

product was examined using HPLC. The correct identity of the PGA deprotection products was also confirmed by co-injection of an equal amount of the product and the reference compound, Tyr(Bzl)-OH, deprotected using TFA (data not shown: HPLC retention times and λ_{max} values were 10.9 min at 228 and 275 nm and 6.4 min at 226 and 274 nm, respectively).

Synthesis of Tyr(Bzl)-Gly-Gly-Phe-Leu-OH. The pentapeptide was prepared using conventional Boc solid-phase chemistry.[1,6] HMBA-AM resin (30 mg, 30 μmol) was derivatized with N-Boc-Leu-OH as described above. Reactions were carried out at room temperature using a stream of dry N_2 to provide agitation.

(a) PGA deprotection: To the Boc-peptide resin was added PGA-P (SSb 50% in propylene carbonate, 30 mM, 600 μmol) in DCM (20 mL). A UV lamp (365 nm) was directly pointed toward the reaction vessel and turned on for 20 min. The solution was then left for an additional 8 min and drained. After deprotection, a ninhydrin test was performed to confirm the presence of free amino groups. The resin was extensively washed with DCM, TEA (10%) in DCM, DMF, acetonitrile, and DMF/DCM. Deprotection reactions were also carried out using the PGA-P/photosensitizer system using the same conditions as described above for monomer deprotection. The reaction was monitored, and the resin was treated as described above.

(b) TFA deprotection: To the Boc-Leu resin was added TFA in DCM (40%, 4 mL), and the reaction continued for 30 min. The reaction was monitored and the resultant resin was treated as described above.

(c) Coupling reaction: To the deprotected Leu-HMBA-AM resin was added Boc-Gly-OH (15.8 mg, 90 μmol) in DCM/DMF, DIC (14 μL, 90 μmol), and HOBt (13.8 mg, 90 μmol). The coupling reaction was continued for 2 h and repeated if the ninhydrin test gave positive amino group reading. Upon completion of the reaction, the resin was washed with DCM/DMF. The subsequent coupling steps were carried out as described using an appropriate Boc-amino acid.

(d) Analysis: The coupling and deprotection steps were repeated until the completion of the pentapeptide synthesis. The sequence was cleaved from resin using NaOH (1.0 M in DMF) and neutralized. The sample was injected onto HPLC (Figure 2), and the retention times were identical for the peptide synthesized using either PGA or TFA in the deprotection step. Synthesis yields were calculated from the integration of HPLC peaks monitored at 230 nm and eluted before 80% CH_3CN (Figure 2). The total yield was 84% and the stepwise yield was 96% for both the PGA and TFA synthesis.

The pentapeptides synthesized using either the PGA or the TFA deprotection were purified using semipreparative HPLC. The purified sample was dried and dissolved in DMSO-d_6 (0.5 mL). One- and two-dimensional spectra were recorded. The two sets of peptides produced identical resonances. ^{1}H NMR (DQF−COSY): δ 0.84 (d, 3, J = 6.86), 0.90 (d, 3, J = 6.86), 1.53 (m, 1), 1.54 (m, 1), 1.63 (m, 1), 2.75 (dd, 1, J = 9.95, 13.71), 2.83 (dd, 1, J = 8.62, 14.15), 3.03 (d, 2, J = 13.49), 3.63 (dd, 1, J = 5.53, 16.37), 3.75 (dd, 2, J = 5.75, 17.25), 3.83 (dd, 1, J = 5.52, 17.03), 3.95 (t, 1, J = 5.98), 4.20 (m, 1), 4.56 (m, 1), 5.07 (s, 2), 6.97 (d, 2, J = 8.85), 7.18 (d, 3, J = 7.96), 7.26 (m, 4), 7.33 (t, 1, J = 7.30), 7.39 (t, 2, J = 7.96), 7.44 (d, 2, J = 7.96), 8.07 (d, 1, J = 7.96), 8.11 (t, 1, J = 6.19), 8.32 (d, 1, J = 7.07), 8.72 (m, 1). Specific assignments are given in Table S1. Mass analyses (ESI) of the PGA peptide: calculated for $C_{35}H_{43}N_5O_7$ 645.32, found 645.46.

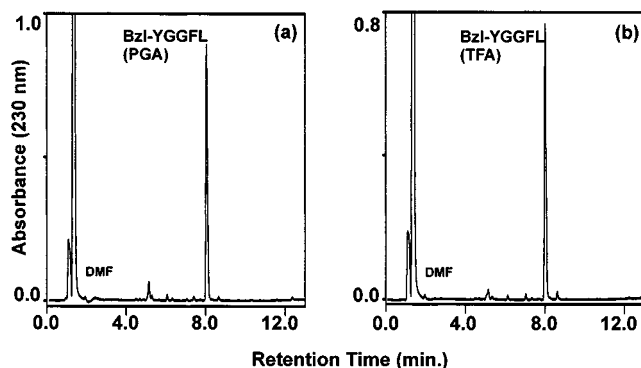
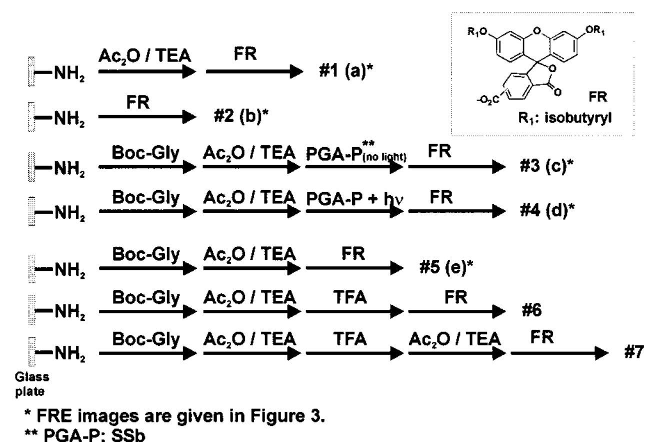


Figure 2. Pentapeptide Tyr(Bzl)-Gly-Gly-Phe-Leu synthesized using either (a) the PGA or (b) the TFA deprotection and the Boc peptide chemistry. HPLC analysis gave a 84% overall yield and 96% stepwise yield in both synthesis.

Scheme 3. Peptide Synthesis on Glass Plates (Table 2)



Dipeptide Synthesis on Glass Plates (Scheme 3). Microcover glass slides (22 × 22 mm^2) positioned in a Teflon holder were cleaned with piranha solution[7] (50:50 v/v H_2SO_4 and H_2O_2) for 1 h at room temperature. After thoroughly rinsing with double filtered water and 95% ethanol, the glass slides were treated with 3-aminopropyltriethoxysilane (3%, NH_2-linkers) in 95% ethanol for 15 min (the linker solution was prepared and stirred 10 min prior to contact with the glass plates). The glass slides were then dipped in ethanol, dried with extra dry N_2, and cured at 100 °C under N_2 for 1 h. The cured slides were cooled to room temperature and washed with 95% ethanol to remove residual silane reagent. The water contact angles of the NH_2-derivatized plates were 70 ± 2° (advancing) and 40 ± 2° (receding). These values are in general agreement with those reported in the literature.[8]

The glass plates derivatized with a monolayer of NH_2-linkers were subdivided into blocks of 3 × 3 mm^2 area, which were contained in plastic tubes for further reaction. The coupling reaction solution containing an appropriate Boc

Table 2. Peptide Synthesis on Glass Plates

glass plate no.	successive reaction steps	purpose of the experiment	FR image
1 (a)[a]	Capping, FR coupling.	Capping surface NH_2 group. Negative FR emission (FRE) indicates no nonspecific FR binding to the surface containing linker.	−
2 (b)[a]	FR coupling.	Demonstrating coupling to linker sequences.	+
3 (c)[a]	Boc-Gly coupling, capping, treatment with SSb (no light), FR coupling.	Negative FRE indicates light is required for PGA deprotection.	−
4 (d)[a]	Boc-Gly coupling, capping, treatment with SSb (light illumination), FR coupling.	PGA deprotection and dipeptide synthesis.	+
5	Boc-Gly coupling, capping, FR coupling.	Capping surface NH_2 group. Negative FR emission (FRE) indicates no nonspecific FR binding to the surface containing peptide monomer.	−
6	Boc-Gly coupling, capping, TFA deprotection, FR coupling.	TFA deprotection and dipeptide synthesis. This is a control reaction.	+
7	Boc-Gly coupling, capping, TFA deprotection, capping, FR coupling.	Capping surface NH_2 group. Negative FR emission (FRE) indicates no nonspecific FR binding to the surface containing amino acids.	−

[a] FRE images are given in Figure 3.

protected amino acid (3 μmol, 30 mM in DMF), as described above, was activated for 20 min prior to adding to the glass plate, and the reaction on glass surface was continued for 1 h. Glass plates were rinsed with DMF and CH_2Cl_2 and subjected to capping with acetyl group. The reaction was carried out for 1 h after adding acetic anhydride (47 μL, 0.5 mmol) and TEA (70 μL, 0.5 mmol) in DMF (150 μL). Deprotection of the surface Boc group using either PGA or TFA deprotection was performed as described above. The plates were subsequently washed with TEA (10%) in DCM and DCM/DMF and ready for the next step coupling reaction.

Fluorescein (FR) Coupling Reaction. To glass plates containing terminal NH_2 group was added a mixture of 4(5)-carboxyfluorescein diisobutyrate (0.23 mg, 0.45 μmol) (Scheme 3) and Boc-Gly-OH (0.45 mg, 2.55 μmol), HOBt (0.46 mg. 3 μmol), and DIC (0.47 μL, 3 μmol) in DCM/DMF (100 μL) (solution preactivated for 20 min prior to reaction). The coupling reactions continued for 1 h, and the glass plates were washed as described above. A summary of the FR coupling reactions on glass plates is provided in Table 2.

Imaging of Glass Plates. The glass plates containing FR were treated with ethylenediamine (50% in absolute EtOH, 100 μL) for 15 min, followed by washing with EtOH and acetone and drying using dry N_2. The plates were placed on a microscope slide under a cooled CCD camera (Apokee Instruments). The fluorophore was excited and detected at 494 and 525 nm, respectively. The fluorescent images of the plates were acquired, processed, and analyzed using the Image Pro program (Media Imagenics). Representative images are displayed in Figure 3.

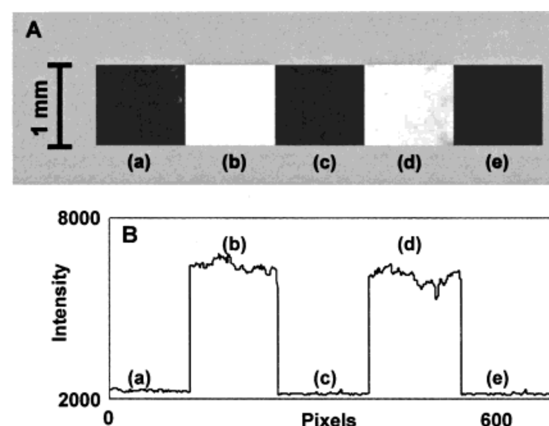


Figure 3. FRE images of the peptide synthesis on glass plates (Schemes 3 and 4, Table 2). (A) Parts a−d are images of experiments 1−4 shown in Scheme 3, and the corresponding interpretation of these results are given in Table 2. Part e is an image of a plain glass plate and is a negative control. (B) The corresponding intensity slices of the FRE images (a−e) shown in panel A.

Results and Discussion

Monomer Deprotection Using PGA. In this work, the suitability of PGA deprotection[9] of N-*t*-Boc (Boc) amino acids used in the popular Boc peptide chemistry (Scheme 1) was first investigated. The requirement is that a PGA remove the protecting group as efficiently as TFA does without causing side reactions and inhibitory effect on the subsequent synthesis. Previously, it was demonstrated that the side chain O-t-Boc group of amino acids can be removed using PGA treatment.[9b] It remains to be tested whether suitable reaction conditions can be developed and incorporated into a multistep synthesis of peptides in solution.

Our first set of experiments established on resin monomer deprotection using a stoichiometric ratio of PGA in DCM. In all experiments described below, PGA and TFA deprotections were performed in parallel with the TFA product serving as the reference. In a regular peptide synthesis, the Boc group on NH_2 of the terminal residue is removed using TFA/DCM. For PGA deprotection, Boc-Tyr(Bzl)-OH was used as a model molecule, which was either free in solution or immobilized on HMBA-AM resin. After cleavage of the product from resin, the results of the deprotection reactions were conveniently analyzed using reverse-phase HPLC. In a typical reaction, PGA precursor (PGA-P) SSb[5a] (Scheme 2) was reacted with the Boc protected amino acid in DCM. The SSb concentration was in the range of 5−30 mM, which

was far less than that of TFA (40% = 5.1 M). When the reaction mixture was not illuminated with UV light, no deprotection was observed based on HPLC analysis. Upon UV illumination, the Boc group was removed to give clean Tyr(Bzl)-OH as the product (Figure 1). As shown in Table 1, the deprotection reaction is a function of PGA-P SSb concentration and light illumination time. These reactions reveal that complete Boc deprotection required a minimal 16 min. Lower concentrations (e.g., 40 mM versus 90 mM) of SSb, however, were more efficient in the initial stage of the deprotection reaction. These PGA deprotection reactions generated clean products (Figure 1) within the time that was quite comparable to that of conventional TFA deprotection in peptide synthesis.

On the basis of the success of monomer deprotection, additional PGA systems were investigated, allowing for further applications. To be useful for the parallel synthesis of addressable peptide arrays, the PGA deprotection chemistry should be both selective so not to cause photolytic side reactions and versatile enough to be compatible with various optical devices used for photoreactions. To meet these requirements, several PGA-Ps or PGA systems, especially those that can be excited at wavelengths longer than 400 nm, were investigated. Some PGA compounds, such as an ISb (Scheme 2) and other onium salts,[9] exhibit the suitable acidity for Boc deprotection but are limited to shorter wavelength excitation (<366 nm). These reagents, however, can easily undergo electron-transfer reactions at triplet excited states in the presence of photosensitizers to give H^+ and photolytic products.[10] In one example, PGA-P ISb (15 mM) and photosensitizer thioxanthenone, in ratios varied from 1:1 to 1:100 in DCM, were reacted with Boc-Tyr(Bzl)-HMBA-AM. The reaction mixture was UV illuminated at 405 nm for 30 min, and the resin was washed and then treated with cleavage conditions. The cleaved amino acids were analyzed, and the HPLC analyses showed that the PGA/photosensitizer combination (with a less than 1:10 ratio) removed the Boc group as efficiently as conventional TFA without causing side reactions. These results demonstrate that a PGA-P can be chosen from a range of reagents, allowing solution PGA deprotection to be performed at longer wavelengths (>400 nm). Light illumination at a longer wavelength is more desirable for synthesis of peptides and organic reactions in general, as under these gentler conditions side reactions are less likely to occur.

On Resin Peptide Synthesis Using PGA. Leucine enkephalin, (Tyr-Gly-Gly-Phe-Leu), an endogenous opioid pentapeptide, was chosen as the first target molecule to be synthesized using the PGA deprotection and standard Boc peptide chemistry.[2d] This compound is commercially available from Sigma, and thus, standard comparisons can be made easily. The syntheses were performed in vessels for manual solid-phase peptide synthesis using the HMBA-AM resin (30 μmol). The coupling reagents and solvents were employed according to standard procedures[2d] except that SSb and light illumination rather than TFA were applied in the Boc deprotection step. After coupling Leu on to the resin, SSb (20 equiv) in DCM was added to the reaction vessel and illuminated with UV light for 16−30 min. The deprotection was confirmed by positive ninhydrin tests and the coupling of Boc-Phe-OH, after extensive washing with TEA/DCM, DCM, and DMF. The PGA deprotection and coupling steps were repeated until the pentapeptide (Tyr(Bzl)-Gly-Gly-Phe-Leu) was synthesized. In a control experiment, the same pentapeptide was synthesized using TFA deprotection. The HPLC analyses of the pentapeptides cleaved from resin demonstrated high quality and high stepwise yield products (Figure 2). The pentapeptides were further examined by NMR and mass analysis, confirming the correct identity of the peptides synthesized using PGA chemistry in the deprotection step.

Peptide Synthesis on Glass Plates. The PGA chemistry described above was tested for peptide synthesis on glass plates, a substrate that will be used in our automated peptide microarray synthesis. The goal of these experiments was to transport the PGA Boc deprotection chemistry to a glass plate surface on which a monolayer of linkers (aminopropylsilane) was immobilized. The glass plate synthesis was optimized, and the process was monitored using fluorescence imaging. These experiments were designed to establish procedures so that no nonspecific adhesion of FR on glass surfaces occurred and that the stepwise synthesis of the peptide monolayer could be monitored.

In a typical experiment, a glass plate of ~9 mm^2 area containing <9 pmol of derivatized NH_2-linkers was used in each reaction. Details of deprotection using PGA or TFA and coupling reaction conditions are described in the Materials and Methods section. Specific experiments performed were (Table 2, Scheme 3) as follows: (a) FR monomer was reacted with an inactive surface (i.e., linker or terminal amino acid NH_2 group was capped with acetyl). Negative FRE indicated the absence of nonspecific covalent attachment of FR (i.e., artifacts) to the glass surface (Figure 3a). (b) Fluorescence emission (FRE) from FR, which was coupled to the terminal NH_2 in the same way as that of an amino acid monomer, was used to monitor the deprotection reaction. The rationale was that if the Boc deprotection were effective, the amino groups released would subsequently couple with the added monomers, such as FR, to form an amide bond. Therefore, FRE is an indicator of Boc deprotection, assuming the coupling step was quantitative (Figure 3b,d). (c) PGA-P SSb (15 mM, 15 μmol) in DCM (1 mL) reacted with the Boc amino group on glass surface without light illumination. FR failed to couple with the growing chain, shown by negative FRE (Figure 3c). (d) For the same reaction as in c but with light illumination, the subsequent coupling of FR resulted in peptide bond formation, shown by positive FRE (Figure 3d). Experiments c and d in comparison prove that the deprotection, as designed, is a light-induced reaction. The (e) image shown in Figure 3 is a control for negative FR. Furthermore, FR was shown not to stick to the surface of peptide monomer (experiments 5 and 7, Scheme 3 and Table 2). These experiments also indicate that capping by the acetyl group is quantitative. TFA deprotection experiments were carried out in parallel to those using a PGA (experiment 6, Scheme 3 and Table 2), and comparable results were obtained (data not shown). These experiments established basic procedures for PGA controlled

peptide synthesis on glass plates and FRE monitoring of the synthetic efficiency.

Conclusions

The PGA reactions described herein for amino acid N-*t*-Boc group deprotection and peptide synthesis are further examples illustrating the diverse applications of the solution PGA chemistry. A stoichiometric quantity of PGA resulted in quantitative cleavage of the N-*t*-Boc group to give high quality products within a time comparable to conventional deprotection reaction. These reactions may serve as a control step in combinatorial synthesis using otherwise conventional reaction conditions. Previously, we demonstrated the chemistry applied to the deprotection of the DMT-OR (DMT: 4,4′-dimethoxytrityl; R: an organic moiety) to yield HOR products.[5] The reactions shown here demonstrate the chemistry applied to the deprotection of the Boc-NHR group to yield NH_2R products. Once these functional groups are selectively deprotected using light, a variety of derivatives can be made by reacting with the deprotected OH or NH_2 groups. The PGA controlled selective deprotection and coupling steps are repeated to produce combinatorial molecules. In principle, these reactions differ from the photoresist/polymeric chemical amplification reactions used in microfabrication[9] and deprotection of the DMT protected nucleotides.[11] The PGA deprotection reactions shown here are performed in conventional solution rather than in solid phase, and they employ various photogenerated reagents instead of being limited to a system of chemical amplification of H^+ (i.e., a self-catalyzed process for generation of H^+). In practice, the solution PGA chemistry avoids the steps of polymer coating, heating, and stripping required by the photoresist chemical amplification reactions.[9] When applied to microarray synthesis, the solution PGA chemistry should overcome the problems associated with the reported light-controlled peptide synthesis,[2e] since product purity will be higher (comparable to conventional peptide synthesis) and there is no need for photolabile group protected amino acid monomers. The principle of the PGA chemistry demonstrated here is also applicable to peptide synthesis using photogenerated base (yet to be developed) and the Fmoc chemistry. These methods are likely to be best utilized in the automated parallel synthesis of focused (scope-limited) libraries, which would require composition variation in a limited number of residues so that synthesis can be completed in a short period of time. Presently there are strong demands for small peptide libraries containing sequences of eight residues or less with two or three combinatorial residues for applications such as screening antibodies and specific binding proteins. Our effort is underway to establish an integrated chemistry and instrument to permit efficient generation of addressable small peptide libraries on an automated, flexible, and cost-effective microarray platform.

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Supporting Information Available. Specific ^{1}H assignments for the YGGFL pentapeptide (Table S1). This material is available free of charge via the Internet at http://pubs.acs.org.

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