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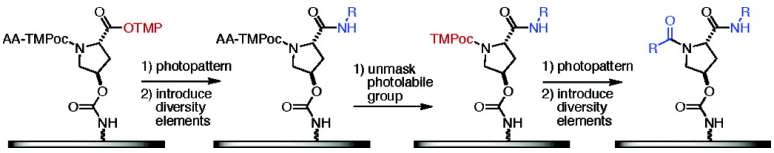

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Light-Directed Radial Combinatorial Chemistry: Orthogonal Safety-Catch Protecting Groups for the Synthesis of Small Molecule Microarrays

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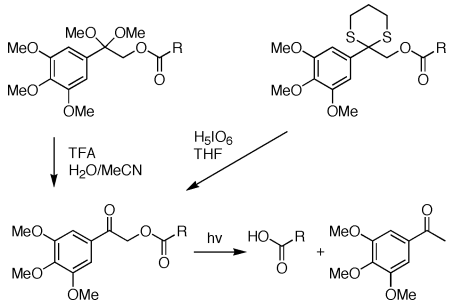
Methods for in situ light-directed synthesis have enabled the production of linear polymers in spatial arrays, including peptides,[1,2] oligocarbamates,[3] oligonucleotides,[4,5] and peptoids.[6] The polymers are assembled through iterative unmasking of reactive groups[1,5] and monomer coupling cycles. The locations of products in the array are controlled by photolithography using physical masks, or more flexibly, by light reflected from a digital micromirror device to produce arrays with up to 786 432 features.[7] The creation of small molecule microarrays, by in situ synthesis[8] or through the robotic spotting of compound libraries, allows binding assays to be conducted simultaneously on all members of the small molecule array, greatly reducing the time and cost of high throughput screens.[9] Although chemistries for the synthesis of linear polymers are well developed, the synthesis of small molecule microarrays with radial diversity requires chemistries that allow light-directed chemistry to occur at multiple independent sites on a scaffold. The independent orthogonal deprotection of photolabile protecting groups (PLPG) has been achieved through the use of PLPGs that respond selectively to light of differing wavelengths,[10] although the reported selectivity is not absolute and the number of independent groups that can be developed may be limited by the broad absorption spectra of photolabile protecting groups.

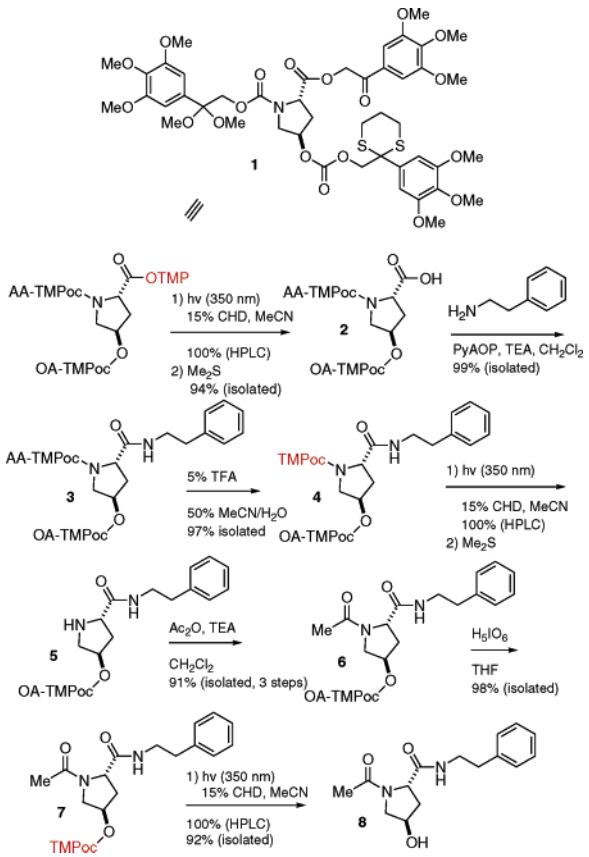
Safety-catch (SC) PLPGs[11] and linkers[12] based on the benzoin[13] PLPG have previously been developed to reduce light sensitivity and improve the chemical stability of PLPGs used in syntheses. Here we report the development of orthogonal safety-catch photolabile protecting groups allowing multiple reactive sites on a scaffold to be independently photodeprotected. We chose to develop safety-catches based on the phenacyl[14] PLPG since masking the carbonyl renders these groups photoinert. Two SC−PLPGs were developed. In the first, the ketone of a 3,4,5-trimethoxyphenacyl group (TMP) is masked as a dimethyl ketal, producing an acid activatible safety-catch (AA-TMP); in the second, the ketone is masked as a 1,3-dithiane, producing an oxidatively activatible safety-catch (OA-TMP). These can be sequentially activated upon conversion of the dimethyl ketal or the dithiane to a photolabile ketone with aqueous acid or periodic acid, respectively (Scheme 1). These two SC−PLPGs, together with the directly photolabile TMP protecting group, provide three independent protecting groups that can be used for light-directed radial combinatorial chemistry.

To determine optimal activation and photolysis conditions for these protecting groups, we synthesized several TMP esters, carbonates, and carbamates and investigated conditions for activation and photolysis in solution (data not shown). These experiments revealed that the photolysis of TMP protecting groups in the presence of 15% 1,4-cyclohexadiene (CHD) to scavenge free radicals gave near quantitative yields in MeCN. Additionally, 0.1% thioxanthone could be added as a triplet sensitizer to allow deprotection at long wavelengths (350−420 nm) in identical yield.[15] Triplet sensitization by thioxanthone required the presence of the 3,4,5-trimethoxy substituents on the phenacyl group *presumably*

Scheme 1. Orthogonal Safety-Catch Protecting Groups Based on the Trimethoxyphenacyl Group



Scheme 2. Solution-Phase Derivatization of a Protected Hydroxyproline Scaffold



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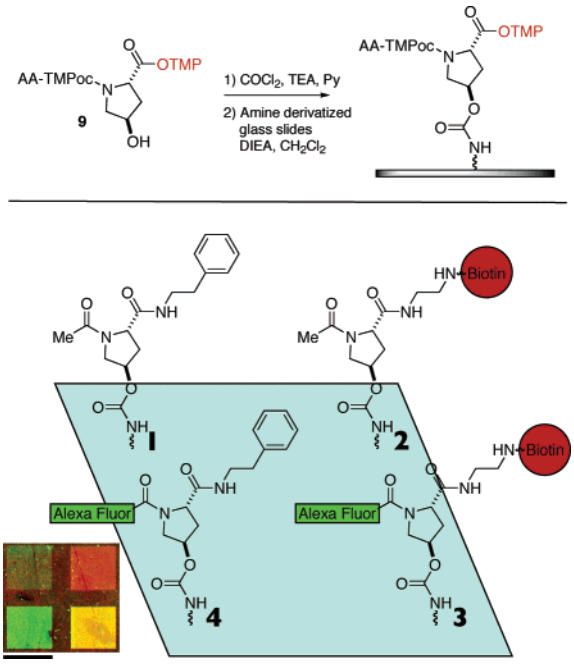

Figure 1. Loading protected hydroxyproline onto glass slide (top), structures of intended array elements (bottom), and fluorescence image of the microarray (inset with 500 μm scalebar).

to sufficiently lower the energy of the triplet state. The dimethyl ketal was hydrolyzed in quantitative yield by 5% trifluoroacetic acid (TFA) in 50% aqueous MeCN, and the dithiane was oxidatively cleaved in near quantitative yield by H_5IO_6 in THF.

To demonstrate the utility of SC−PLPGs for small molecule radial combinatorial chemistry, we synthesized a protected hydroxyproline scaffold **1** with a TMP−ester-protected carboxylic acid, an AA-TMP−carbamate-protected amine, and an OA-TMP−carbonate-protected alcohol (Supporting Information). Photolysis of the TMP ester (Scheme 2) yielded acid **2** in 94% yield (quantitative by HPLC). Dimethyl sulfide was added during workup of the photolysis to scavenge oxidants and protect the dithiane functionality. Coupling with phenethylamine yielded amide **3** in 99% yield. Hydrolysis of the dimethyl ketal gave TMP−carbamate **4** in 97% yield, and photolysis yielded free amine **5** (quantitative by HPLC) that was acylated with acetic anhydride to give **6** in 91% yield. Oxidative cleavage of the dithiane with H_5IO_6 gave the TMP−carbonate **7** in 98% yield, which was photolyzed to give the alcohol **8** in 92% yield (quantitative by HPLC).

To demonstrate the utility of SC−PLPGs for the light-directed synthesis of small molecule microarrays, we covalently attached alcohol **9** to the surface of an amine-derivatized glass slide (Telechem Superamine) and capped any unreacted amines with acetic anhydride (Figure 1). The maskless array synthesizer was used to synthesize a 2 × 2 small molecule microarray in which initial photolysis of the TMP ester in array elements 2 and 3 produced free carboxylic acids that were coupled to biotin−ethylenediamine (Pierce). Array elements 1 and 4 were then photolyzed, unmasking carboxylic acids that were coupled to phenethylamine with DIC/HOAt. The AA-TMP safety-catch protecting groups were activated with 5% TFA in 50% aqueous MeCN, photolyzed to unmask secondary amines in array elements 1 and 2, and acetylated with acetic anhydride. Array elements 3 and 4 were then photolyzed and derivatized with Alexa-Fluor (488) NHS ester (Molecular Probes) to generate amides. The slide was incubated with the streptavidin−Texas Red conjugate (Amersham Biosciences) and scanned to reveal the positions of biotin and Alexa-Fluor functionalities (Figure 1). The image reveals the anticipated pattern of fluorescence from the intended array elements.

We have demonstrated the utility of SC−PLPGs to enable the light-directed synthesis of a small molecule microarray with radial diversity elements. The incorporation of diversity elements at branching points on scaffold molecules will greatly expand the scope of materials that can be synthesized in a light-directed manner. We expect that the synthesis of small molecule microarrays using the maskless array synthesizer will significantly reduce the costs of library construction and screening and enable the identification of small molecules with useful biological activities. Additionally, this chemistry could be applied to the in situ synthesis of sensor arrays.

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Supporting Information Available: Synthetic protocols and characterization of compounds **1**−**9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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