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Continuous Photochemical Cleavage of Linkers for Solid-Phase Synthesis

Mattan Hurevich,[†] Jeyakumar Kandasamy,[†] Bopanna M. Ponnappa,[†,‡] Mayeul Collot,[†] Daniel Kopetzki,[†] D. Tyler McQuade,[†,§] and Peter H. Seeberger*[,†,‡]

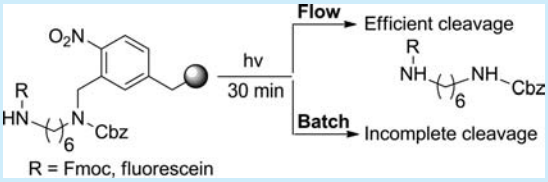
[†]Max Planck Institute of Colloids and Interfaces Am Mühlenberg 1, 14476 Potsdam Germany

[‡]Institute of Chemistry and Biochemistry, Freie Universität Berlin, Arnimallee 22, 14195 Berlin Germany

[§]Department of Chemistry and Biochemistry, Florida State University, Tallahassee, Florida 32306, United States

Supporting Information

ABSTRACT: Photolabile linkers are an attractive alternative for solid-phase synthesis because they can be cleaved using light. However, irradiation in a classical batch photoreactor results in incomplete cleavage of the photolabile linkers. It is demonstrated that a continuous flow photoreactor is superior to a batch photoreactor for the cleavage of a linker from polystyrene resin.



Solid-phase organic chemistry (SPOC) relies on the presence of readily cleavable linkers to join the resin and molecule of interest.[1] Once the sequence of reactions on solid support is complete, the product must be cleaved from the resin without destroying the synthesized compound. Photolabile groups are often stable to many reagents and reaction conditions and can be cleaved under mild conditions. Photocleavable linkers consist of a photolabile functional group which, when exposed to light of an appropriate wavelength, undergoes photoinduced fission to liberate the linker with the desired synthesized substrate.

While many photocleavable linkers have been reported, their application in SPOC is not common.[2] A factor limiting their use is that cleavage in traditional batch photoreactors is inefficient because of the presence of a fixed light source resulting in low penetration of light to the entire sample and the presence of an insoluble solid support solution, resulting in a heterogeneous mixture. In addition, cleavage in a batch photoreactor requires an extended reaction time and cannot easily be scaled up.

On the other hand, continuous flow reactors have proven efficient in performing challenging photochemical reactions.[3] The use of flow photoreactors to induce cleavage from a solid support is an attractive alternative to batch synthesis because the solid support will receive a more homogeneous exposure to the light and scaling up reactions in flow is typically straightforward. Indeed, we have previously observed that irradiation in flow, where beads are pumped through tubes wrapped around a light source, results in efficient cleavage of the product from the resin.[4]

A number of mechanistic models can be suggested to explain the improved yields observed for flow photocleavage, but systematic experimental evidence has not yet been described. Here, cleavage efficiency in batch and continuous flow reactors was compared. Polystyrene beads, labeled with either Fmoc or fluorescein, were used to study the factors influencing the cleavage mechanism in both reactors. Our study demonstrates the advantage of using a flow reactor for photocleavage reactions and highlights the limitations of the classic batch reactor.

Two photoreactors were constructed to compare cleavage efficiencies in batch and in flow (Figure 1). The batch reactor we used is representative of a popular apparatus used for photocleavage. The continuous-flow setup was a modified version of the previously reported photoreactor regularly used for continuous-flow photosynthesis.[3]

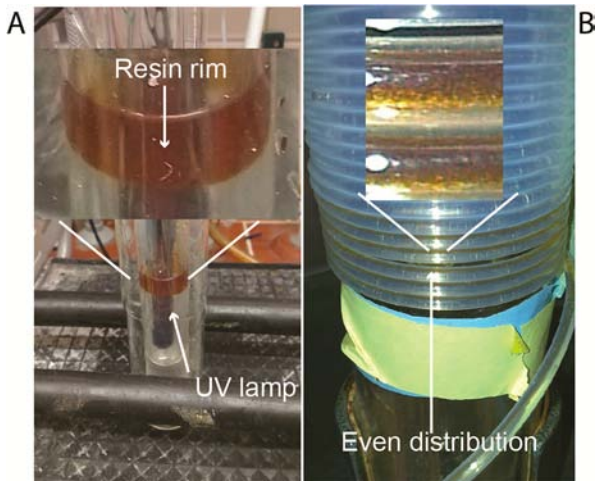


Figure 1. Resin distribution in photoreactors: (A) in batch photoreactors resin packs as a thick rim around the light source as there is no movement; (B) constant flow helps to distribute the resin in flow photoreactors.

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To evaluate irradiation in batch, a classical photoreactor was constructed from a double-jacketed glass reactor with a constant cooling water circulation as the basic setup. To cleave the compound from the support, the polystyrene resin (suspended in DCM) was placed in the reactor central well. To block irradiation below 300 nm, a Pyrex filter was placed between the light source and reaction well. A UV pencil lamp (365 nm) was inserted into the Pyrex filter and adjusted to reach the level of the resin (as shown in Figure 1A). The entire reactor was covered with aluminum foil and placed on an oval shaker. Irradiation was performed for 30 min with constant shaking of the resin to achieve maximal exposure. In order to understand the effect of shaking on the reaction, the same photocleavage procedure was repeated without shaking. After irradiation, the light source was turned off, and the aluminum foil was taken off. The Pyrex filter and the lamp were removed, and the solid support was taken out using a Pasteur pipet and filtered through a polypropylene filter tube equipped with polyethylene frit (PE filter) to give the crude compound.

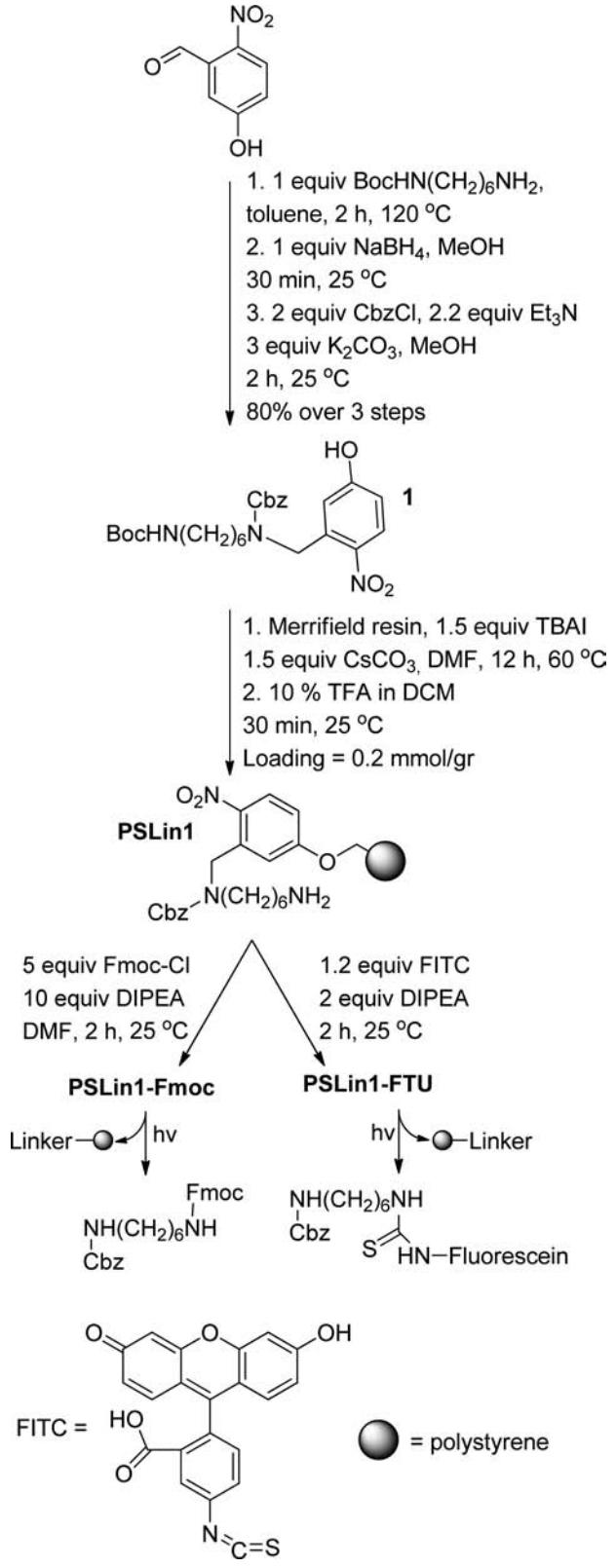
The flow reactor was built in a very different fashion (Figure 1B). The entire setting was placed in a sealed aluminum box equipped with entry and exit holes for the fluorinated ethylene propylene (FEP) tubing, the electrical cords, and the water supply lines. Based on a previous setup by Hook et al.,[3a] a medium-pressure mercury lamp (450 W) was covered with a Pyrex filter, and both were inserted into a double-jacket immersion well with water circulation. FEP tubing was wrapped around the jacket so that the entire length of the lamp was covered. Both ends of the FEP tubing were connected outside the box, with one end connected to a syringe pump and the other end to a flask fitted with a PE filter, to separate the resin from the filtrate (see pictures in the Supporting Information). In a typical experiment, the solid support was preswollen in DCM and pushed through the FEP tubing using a syringe pump (usually in a vertical position so that the floating resin can easily pass into the tube) with a residence time of 30 min before being filtered through the PE frit. The tubing was cleaned by injection of a fresh solution of DCM through the system before the next use.

The *o*-nitrobenzyl photolabile group was used as the basis for a model linker to study the cleavage mechanism.[5] An amine-functionalized *o*-nitrobenzyl-based linker (**1**) was synthesized on the basis of a previous procedure.[4b]

The photolabile linker **1** was synthesized by forming first a Schiff base between 5-hydroxy-2-nitrobenzaldehyde and *N*-Boc-1,6-hexanediamine. Reduction of the imine with $NaBH_4$ gave a secondary amine that was later protected using CbzCl under basic conditions to give **1** (Scheme 1).

Merrifield resin (chloromethyl polystyrene) was functionalized using **1** in the presence of tetrabutylammonium iodide and $CsCO_3$ in *N,N*-dimethylformamide (DMF). The Boc protecting group was subsequently removed using TFA to give the solid support **PSLin1**. **PSLin1-Fmoc** and **PSLin1-FTU** (FTU, fluorescein thiourea) were synthesized by coupling the free amine of **PSLin1** under basic conditions with Fmoc-Cl and Fluorescein isothiocyanate (FITC), respectively (Scheme 1). **PSLin1-Fmoc** was cleaved in flow, and the product was characterized to demonstrate that irradiation does not affect the cleaved compound (see the Supporting Information). Although the photocleavage reactions preceded efficiently, variable quantities of impurities where always present in the crude sample (10−30%). Even though these impurities could be easily removed by chromatography (see the Supporting Information), their presence made evaluation of the cleavage efficiency by crude mass yield inaccurate and too prone to errors, especially when small amounts of resin were used. To overcome the above limitations, the cleavage efficiency of flow and batch reactors was measured using more sensitive optical methods (UV spectrophotometry for **PSLin1-Fmoc** and

Scheme 1. Synthesis and Labeling of the Photolabile Linker



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confocal microscopy for **PSLin1-FTU**) that are accurate even for small amounts of resin. By using optical methods we decreased the error associated with the impurities as we examined only the presence or absence of the fluorophore marker.

Standard Fmoc quantification[6] of **PSLin1-Fmoc** beads before and after irradiation in batch (with shaking) showed that 70% of the Fmoc protecting group was still on the beads and only 30% was cleaved and found in solution. On the other hand, irradiation in flow resulted in a greater than 70% decrease in Fmoc loading of the **PSLin1-Fmoc** beads. Based on these results, we assumed that the efficiency of the cleavage was related to the overall exposure of the beads to the light source during irradiation. This method provided us with an indication that flow cleavage was more efficient than batch. However, additional experiments were designed in an effort to obtain visual evidence that explains the mechanism of cleavage.

A fluorescein thiourea (FTU)-labeled solid-supported photolinker **PSLin1-FTU** (Scheme 1) was used to study the impact of the irradiation methods on the cleavage pattern. Confocal laser scanning microscopy was used to measure and visualize bead fluorescence before and after irradiation.

To ensure irradiation does not influence the fluorescein moiety, aminomethylated polystyrene (commercially available) was loaded with FITC, and the fluorescence was measured before and after irradiation (see the Supporting Information). Our results indicate that irradiation did not significantly quench the fluorescence of the labeled beads when no photolabile linker is used.

Conversely, when fluorescein-labeled beads **PSLin1-FTU** (which has photolabile linker) were irradiated, a measurable decrease in fluorescence was visible. Mean fluorescence intensity (MFI) measurements were used to quantify the loss of fluorescence due to photocleavage of the linker-fluorescein moiety (results summarized in Figure 2). Unlabeled beads (**PSLin1**) were imaged to measure bead autofluorescence as a negative control (Figure 2A), whereas labeled beads (**PSLin1-FTU**) exhibit strong fluorescence and served as a positive control (Figure 2B).

Four different irradiation methods were compared. Beads were irradiated in a standard batch photoreactor with or without shaking the photoreactor using an orbital shaker (Figure 2D ,C) to evaluate the effect of mixing on batch cleavage. Beads were also injected through a small (1.5 mm tube, inner diameter 0.8 mm, Figure 2E) or large diameter tube (3.2 mm tube, and inner diameter 1.5 mm, Figure 2F) to study the contribution of tube diameter on the flow assisted photocleavage.

Batches that were irradiated without shaking (Figure 2C) afforded a heterogeneous mixture where some beads experienced almost complete photocleavage (beads with a dark center and only a rim of fluorescence) while others showed only a slight decrease in fluorescence. We assumed that irradiation without shaking resulted in a nonhomogeneous bead population because those beads positioned in proximity to the light source absorbed most of the energy and shielded others that were located further from the source. In contrast, batch irradiation with shaking (Figure 2D) resulted in a population of beads that mostly displayed partial cleavage of the Lin-FTU and overall significantly decreased fluorescence. Clearly, more beads were exposed to the light source when the reactor was shaken in comparison to the previous irradiation experiment without shaking. Hence, the mixing increased the absolute number of

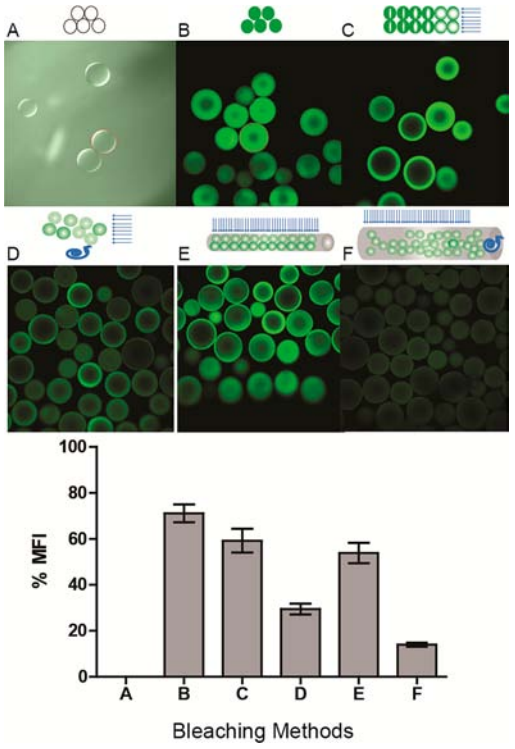

Figure 2. Confocal microscopy images of fluorescein labeled beads (**PSLin1-FTU**): (A) unlabeled beads (**PSLin**); (B) fluorescein-labeled beads (**PSLin1-FTU**); (C) **PSLin1-FTU** beads after irradiation in batch; (D) **PSLin1-FTU beads** after irradiation in batch using an orbital shaker; (E) **PSLin1-FTU beads** after irradiation in flow-reactor using a narrow bore tube; (F) **PSLin1-FTU beads** after irradiation in a flow-reactor using a large bore tube. Mean fluorescence intensity (MFI) analysis (bottom graph) shows that standard batch irradiation of the beads results in minimal cleavage of fluorescein (C) while irradiation in flow using the large bore tube results in almost complete cleavage of fluorescein (F).

photocleavage events from the resin and at the same time increased the homogeneity of cleavage by ensuring that each bead experienced similar light exposure. From these batch experiments, we conclude that both proximity and homogeneous exposure to the light source are crucial for efficient cleavage.

The batch cleavage system incorporating shaking is an improvement to the classic batch reactor but is technically challenging and more dangerous to handle. In addition, it does not address the problem of scaling up the reactions.

To examine how flow affects cleavage, we performed photocleavage experiments in the photoreactor using small and large bore tubes as the flow-through channel.

When **PSLin1-FTU** beads were pumped through small dimension tubing (0.8 mm inner diameter), a heterogeneous population of irradiated beads resulted (Figure 2E). Many beads exhibited fluorescent intensity similar to the positive control while others showed almost no fluorescence. These results indicate that the beads pass through the narrow tubing as a multilayer slug that cannot move freely along the diameter of the tubing. As a result, beads closer to the light source undergo near-complete photocleavag,e while those beads that are on the outside (away from the light source) are shielded.

When a wider tubing (1.5 mm inner diameter) was used, irradiated **PSLin1-FTU** beads exhibit a much more uniform low fluorescence intensity (Figure 2F). Visual inspection of the

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beads flowing through the larger tube indicated that the beads undergo turbulent mixing and tumbling (see the movie in the Supporting Information). We propose that the mixing results in similar exposure of each bead to the light source and thus results in uniform, highly efficient cleavage of the fluorophore from the resin.

In addition to providing a more efficient cleavage, the flow reactor is easier to use than the batch system. In a standard batch reactor, manual placement and removal of beads and reagents from the reactor requires turning off the light source each time and thus limits throughput. The setup of the flow reactor allows for continuous performance without turning off the system. A simple wash between resin deliveries is sufficient to enable multiple runs in the same reactor.

We have demonstrated that a combination of factors unique to the flow photoreactor leads to improved photocleavage. The flow reactor is an alternative to classical photoreactors and offers high throughput and efficient cleavage in a short amount of time. Flow photocleavage renders photocleavable linkers useful for the solid-phase synthesis of many classes of compounds.

■ ASSOCIATED CONTENT

Supporting Information

Detailed description of the reactors and cleavage procedure, linker preparation, and solid support functionalization, methods of analysis, NMR and HPLC characterization of cleaved compounds, and a movie demonstrating bead movement in flow. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: peter.seeberger@mpikg.mpg.de.

Notes

The authors declare no competing financial interest.

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