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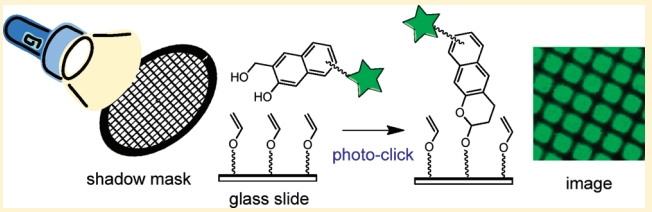
Patterned Surface Derivatization Using Diels−Alder Photoclick Reaction

Selvanathan Arumugam and Vladimir V. Popik*

Department of Chemistry, University of Georgia, Athens, Georgia, 30602

Supporting Information

ABSTRACT: The utility of photochemically induced hetero-Diels−Alder reaction for the light-directed surface derivatization and patterning was demonstrated. Glass slides functionalized with vinyl ether moieties are covered with aqueous solution of substrates conjugated to 3-(hydroxymethyl)-2-naphthol (NQMP). Subsequent irradiation via shadow mask results in an efficient conversion of the latter functionality into reactive 2-napthoquinone-3-methide (*o*NQM) in the exposed areas. *o*NQM undergoes very facile hetero Diels−Alder addition ($k_{D\text{-}A} \approx 4 \times 10^4$ $M^{-1}s^{-1}$) to immobilized vinyl ether molecules resulting in a photochemically stable covalent link between a substrate and a surface. Unreacted *o*NQM groups are rapidly hydrated to regenerate NQMP. The click chemistry based on the addition of photochemically generated *o*NQM to vinyl ether works well in aqueous solution, proceeds at high rate under ambient conditions, and does not require catalyst or additional reagents. This photoclick strategy represents an unusual paradigm in photopatterning: the surface itself is photochemically inert, while photoreactive component is present in the solution. The short lifetime ($\tau \approx 7$ ms in H_2O) of the active form of a photoclick reagent in aqueous solution prevents its migration from the site of irradiation, thus allowing for the spatial control of surface derivatization. Both *o*-napthoquinone methide precursors and vinyl ethers are stable in dark and the reaction is orthogonal to other derivatization techniques, such as acetylene−azide click reaction.



INTRODUCTION

"Click" reactions[1] represent a set of very useful tools in modern chemistry and biochemistry.[2,3] Copper(I)-catalyzed 1,3-dipolar cycloaddition of azides to terminal acetylenes, also known as azide click reaction, became the gold standard of click chemistry and have found applications in many areas ranging from material science[3,4] to chemical biology[5,6] and drug development.[7] While acetylene−azide click chemistry has become commonplace in surface derivatization, as well as polymer and materials synthesis,[3,4] the use of metal catalyst often limits the utility of the method. Recently developed catalyst-free strategies of azide click ligations[8,9] were successfully applied to surface derivatization.[10] "Click" methods based on a Diels−Alder cycloaddition are also gaining popularity since this reaction does not require catalysts, can proceed in high yield under physiological conditions, and does not produce any byproduct.[11] The Diels−Alder click reaction has found applications in material chemistry,[11,12] derivatization of nanoparticles and surfaces with various bioactive molecules,[13] as well as the labeling of oligonucleotides, proteins, and oligosaccharides.[14] However, the generation of a reactive diene−dienophile pair often requires either thermal activation[15] or the use of chemical promoters for the in situ generation of highly reactive dienes.[16]

Light-induced "click" reactions permit spatial control of the immobilization processes. Several photoclick strategies are under development, including cycloaddition of alkenes to photochemically generated nitrile imine,[17] photoinitiated thiol−ene,[18] and thiol-yne[19] reactions, as well as photochemical generation of reactive acetylenes[10b] and uncaging of hydroquinone moieties.[20] We have recently reported a novel photoligation strategy based on very facile hetero-Diels−Alder addition of 2-napthoquinone-3-methides (*o*NQMs) to vinyl ethers.[21] Thus, irradiation of substituted 3-(hydroxymethyl)-2-naphthols (*o*NQM precursor, NQMP, **2**) results in efficient ($\Phi = 0.20$) dehydration of the substrate and the formation of *o*NQM **1** (Scheme 1).[22] In the presence of vinyl ethers (**3**), *o*NQMs undergo very facile ($k \approx 4 \times 10^4$ $M^{-1}s^{-1}$) and quantitative Diels−Alder cycloaddition to yield photostable benzo[g]chromans (**4**, Scheme 1).[21] The unreacted *o*NQM is rapidly hydrated ($k_{H2O} \approx 145$ s^{-1}) to regenerate NQMP (**2**).[22]

In this report, we demonstrate a novel paradigm in light-directed surface derivatization. Conventional photopatterning relies on selective (using shadow mask or laser) activation of light-sensitive compounds immobilized on the surface, which then reacts with the second component in the dark step. In our approach, surface is photochemically inert, thus simplifying preparation and handling of the substrates, but photoreactive NQMP (**2**) is present in solution. A very short lifetime of *o*NQM (**1**) species prevents their migration from the site of irradiation, allowing for high spatial resolution of derivatization. Additional advantage of this approach is in quantitative regeneration of

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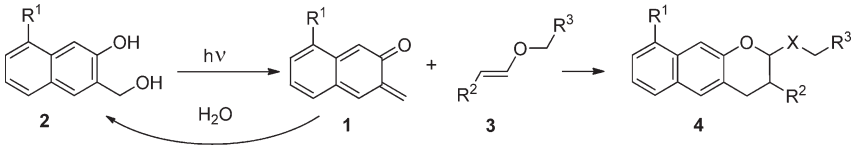
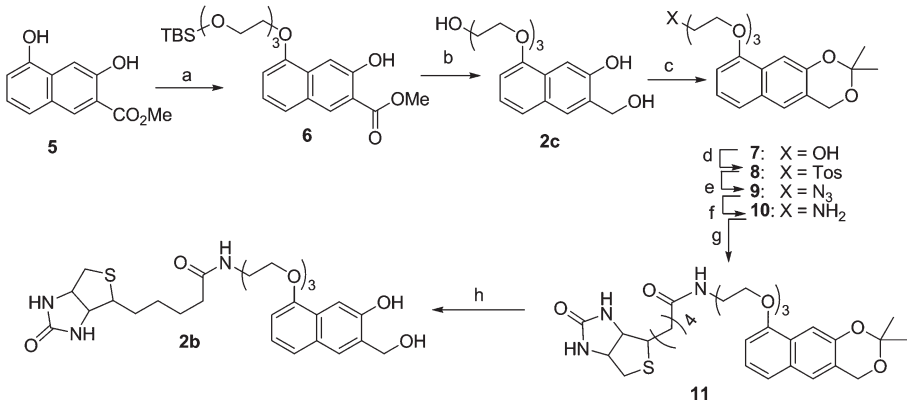
Scheme 1



Scheme 2. Synthesis of NQMP-PEG3-Biotin (2b)[a]



[a] Reagents and conditions: (a) $TBDMS(OCH_2CH_2)_3I$, K_2CO_3, acetone, reflux, 65%; (b) i) $LiAlH_4$, THF; HF/CH_3CN aq., 85% over 2 steps; (c) TsOH, 2,2-dimethoxypropane, acetone,90%; (d) TsCl, pyridine, DCM, 82% ; (e) NaN_3, DMF,60 °C, 80%; (f) $LiAlH_4$, Et_2O, 85%; (g) d-biotin, EDC, DMAP, DMF, 83% ; (h) Amberlyst-15, methanol, RT, 95%.

NQMP by hydration of unreacted *o*NQM molecules. Since only a small fraction of NQMP is consumed in the derivatization procedure, the labeling solution can be reused many times. The addition of oNQMs to vinyl ethers is much faster than conventional click reactions providing an opportunity for the development of time-resolved methods. Finally, competition between hydration and cycloaddition makes oNQMs very selective. Only vinyl ethers are reactive enough to outcompete water. This is a definite advantage over methods involving generation of other reactive species, e.g., radicals, nitrenes, and carbenes.

RESULTS AND DISCUSSION

To illustrate the utility of photochemically induced hetero-Diels–Alder cycloaddition for light-directed surface derivatization, we have patterned fluorescently labeled Avidin onto vinyl ether-derivatized glass slides.

Preparation of Materials. Synthesis of 6-(hydroxymethyl)-naphthalene-1,7-diol–tri(ethylene glycol)-biotin conjugate (NQMP-TEG-biotin, **2b**) is outlined on the Scheme 2. Methyl-3,5-dihydroxy-2-naphthoate (**5**) was prepared according to previously reported literature[23] and was etherified to iodo-TEG-TBDMS, followed by lithium aluminum hydride reduction and deprotection to give NQMP-TEG (**2c**). The glycol moiety was then protected as acetone ketal and TEG terminal hydroxy group was tosylated. Nucleophilic substitution of tosyl ester with azide ion produced **9**, which was further reduced to an amino derivative **10**. EDC-promoted coupling of the latter with d-biotin, followed by ketal hydrolysis produced target NQMP-TEG-biotin (**2b**, Scheme 3).

It is important to note, that synthetic intermediates shown in the Scheme 2 can serve as entry points for the conjugation of NQMP with various "payload" substrates. Thus, molecules inert to hydride reduction can be coupled directly to **5**. NQMP-TEG (**2c**) or its protected analog **7** possess primary alcohol moiety, which is suitable for Mitsunobu coupling or esterification; acetylene click reaction can be employed to modify azide derivative **9**; molecules containing carboxylic acid or NHS-ester groups can be readily coupled with protected NQMP-TEG-NH_2 (**10**).

Light-Directed Surface Derivatization. Vinyl ether derivatization of commercial epoxide-functionalized glass slides was achieved by treating the slides with 2-hydroxyethyl vinyl ether or 6-methoxyhex-5-en-1-ol in the presence of catalytic amount of anhydrous *p*-toluenesulfonic acid. Alternatively, incubation of epoxy slides in 2-(2-(vinyloxy)ethoxy)ethanamine dichloromethane solution yields vinyl ether functionalized glass slides without the need for a catalyst.[24]

The resulting vinyl ether-derivatized slides were covered with aqueous solution of NQMP-TEG-biotin **2b** (1×10^{-4} M), and irradiated using 300 or 350 nm fluorescent lamps. Photochemical dehydration of 3-(hydroxymethyl)-2-naphthol moiety in **2b** produces derivative of *o*NQM, which undergo very facile Diels–Alder cycloaddition to vinyl ether groups on the surface resulting in covalent immobilization of biotin molecules. Unreacted *o*NQM species rapidly react with water to regenerate **2b**. Biotinylated glass slides were then developed with FITC-avidin in PBS solution for 15 min at 2 °C and thoroughly washed (Scheme 3).[24] Surface derivatization was followed by FTIR and contact angle measurements.[24]

Two procedures were employed to achieve patterned immobilization of FITC-avidin on vinyl ether-derivatized slides. In the first method, 2 μL droplets (~1 mm in diameter) of 0.1 mM

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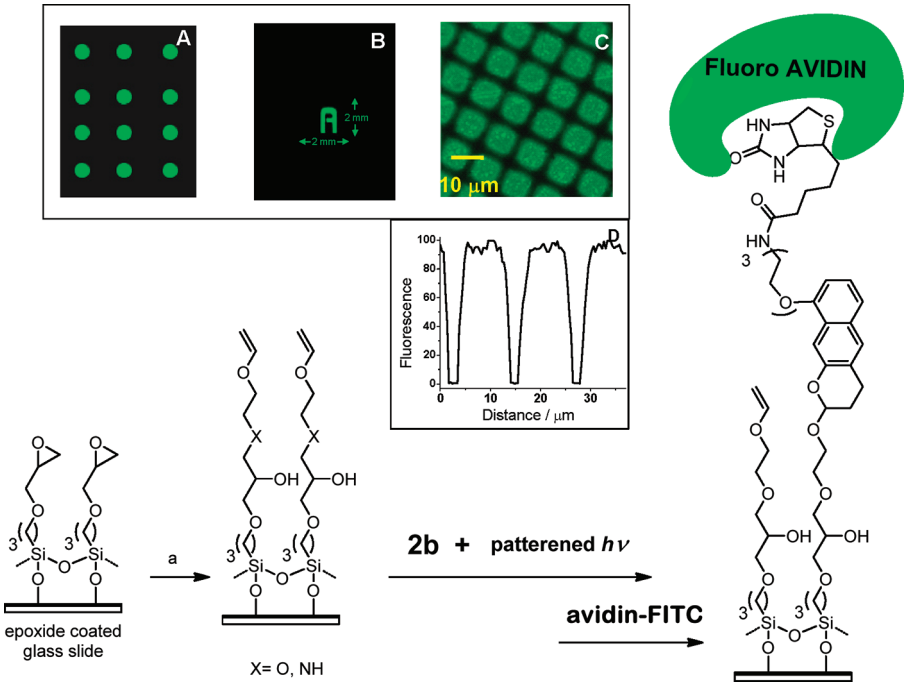
Scheme 3. Schematic Representation of Preparation and Light-Directed Biotinylation of Vinyl Ether-Coated Slides Followed by Immobilization of FITC-Avidin[a]



[a] The insert shows fluorescent images of vinyl ether-coated slides spotted with 2 μL drops of 10^{-4} M NQMP-TEG-biotin aqueous solution and flood irradiated (A); or covered with 10^{-4} M NQMP-TEG-biotin aqueous solution and irradiated via "A" mask (B) or 12.5 μm pitch copper grid (both enlarged). Insert "D" shows fluorescent intensity profile along the line perpendicular to the pattern "C".

NQMP-TEG-biotin (**2b**) aqueous solution were placed on the slides, which were then flood irradiated for 10 min with fluorescent UV lamp, washed, and stained with FITC-avidin[24] (Scheme 3, Insert A). The actual photopatterning has been achieved by the irradiation of the slides covered with solution of **2b** via a shadow mask. The "latent" image was also developed using FITC-avidin (Scheme 3, inserts B and C). We used two different masks for irradiation: first one had ~2 mm high letter "A" cut in a steel plate. For the second, we used 12.5 μm pitch copper grid. As insert C in Scheme 3 illustrates this avidin photopatterning method readily reproduce features as small as 5 μm (the width of the grid bars; Scheme 3, insert D). This is a remarkable result, since the photoreactive compound is not immobilized on the surface but rather present in low viscosity solution. High resolution photopatterning in this case relies on a short lifetime ($\tau \approx 7$ ms) of *o*NQM in aqueous solution, which prevents its migration from the site of irradiation.

To evaluate the efficiency of photochemical surface derivatization, vinyl ether-coated slides covered with 0.1 mM aqueous solution of NQMP-TEG-biotin (**2b**) and irradiated for various periods of time. Slides were then washed, treated with FITC-avidin, and fluorescence intensity was recorded. The relative fluorescence intensity of photoderivatized slides increased with the exposure time reaching saturation at approximately 10 min. Longer irradiation times have virtually no effect on the fluorescence of the slides (Figure 1). The vinyl ether-derivatized slide incubated for 30 min in solution of **2b** in the dark shows no detectable Fluorescein emission (0 min, Figure 1).

To test the photostability of photochemically induced linkage, two slides were biotinylated using 10 min irradiation in 0.1 M **2b**

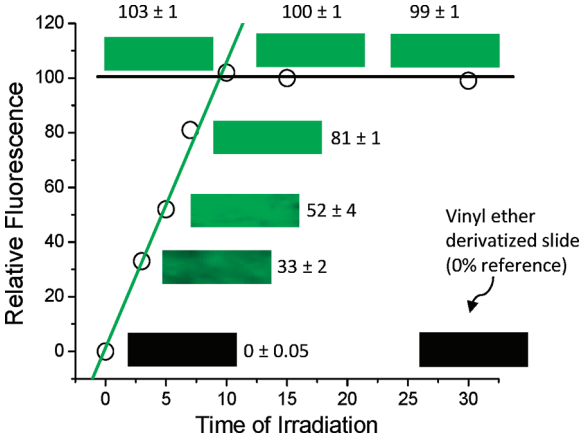


Figure 1. Relative fluorescent intensity of FITC-avidin-stained vinyl ether-derivatized glass slides after various time of irradiation in 0.1 mM NQMP-TEG-biotin (**2b**) solution. Numerical values of average fluorescent intensity of the slide are shown next to each image.

solution and washed. One of the slides was further irradiated in aqueous PBS solution for 30 min. Both slides were then developed with FITC-avidin solution. The fluorescent intensity of the irradiated slide was 99% of the control one. This experiment confirms that the formation of Diels–Alder adduct on the surface is photochemically irreversible. Since concentration of the NQMP-TEG-biotin (**2b**) in the solution remains virtually unchanged after photolysis, saturation of fluorescence after 10 min of irradiation indicates that all accessible vinyl ether groups on the surface have reacted with *o*NQMs. Growth of

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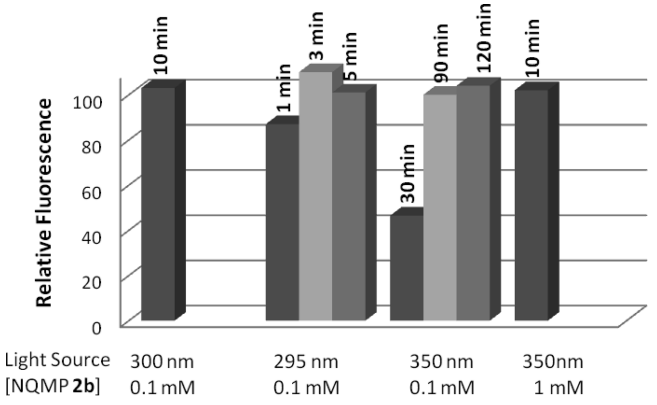

Figure 2. Relative fluorescent intensity of FTIC-Avidin-stained glass slides biotinilated under different conditions.

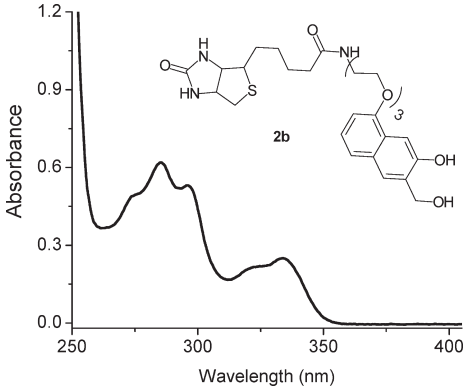

Figure 3. UV-spectrum of 0.05 mM aqueous solution of NQMP-TEG-biotin (**2b**).

fluorescence intensity follows zeroth-order kinetics (Figure 1), indicating that the formation and diffusion of *o*NQM to the surface, rather than cycloaddition to immobilized vinyl ether groups, is a rate-limiting step in the derivatization process.

It is important to note that all experiments shown discussed above (Scheme 3C and Figure 1) were conducted using the same solution of **2b**. Since the amount of the substrate required to derivatize a monolayer is so minute, no detectable changes of concentration of NQMP **2b** were detected by UV measurements in the course of photobiotinylation experiments. HPLC analysis shows no significant accumulation of byproducts even after eight photolyses, underscoring the cleanness of the photodehydration–hydration reaction of **2b**.

The irradiation time can be adjusted using different light sources or concentrations of NQMP. Medium pressure mercury lamp equipped with 295 nm cut off glass filter allows us to cut exposure time to 3 min at 0.1 mM NQMP **2b** (Figure 2). However, if longer wavelength irradiation is required, 350 nm light can be employed for patterning. This process, however, is less efficient. Using 350 nm lamps with intensity similar to 300 nm fluorescent tubes discussed above, the complete fictionalization is achieved at ~100 min of irradiation. The reduced rate of surface derivatization is, apparently, due to the lower extinction coefficient of NQMP **2b** at 350 nm (Figure 3). In fact, at 10-fold higher concentration of **2b** (1 mM), complete conversion at this wavelength is achieved in 10 min (Figure 2).

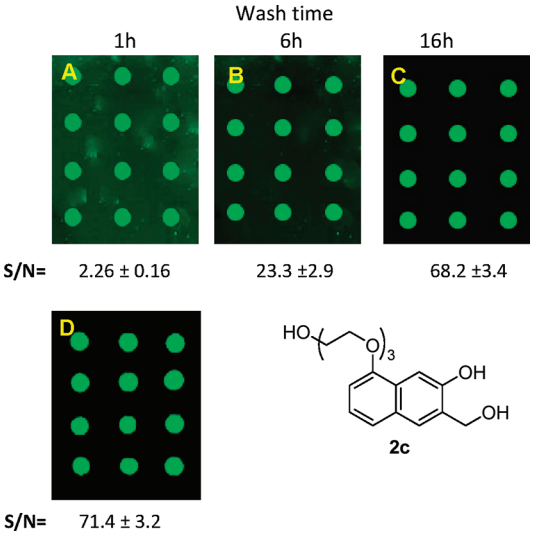

Figure 4. Vinyl ether-derivatized slides A–D were spotted with NQMP **2b** solution and irradiated with 300 nm light. Slide "D" was further irradiated in NQMP **2c** solution. Slides were stained with FTIC-Avidin and washed in PBS solution for h (A and D), 6 h (B), and 16 h (C). Average signal-to-background fluorescent intensity ratios are shown under each image.

Protein patterning procedures often require extensive washing to remove substrate nonspecifically absorbed on the surface. In our experiments, the FITC-Avidin-stained surfaces (e.g., A and B, Scheme 3) were washed by sonicating the glass slides in PBS solution for 30 min followed by overnight incubation in fresh phosphate buffer. From practical point of view, a shorter washing procedure could enhance the efficiency of photoclick protein patterning. In order to reduce nonspecific protein binding, we have developed a photochemical PEGylation procedure. After the initial patterning of the substrate on the vinyl ether-derivatized surfaces, slides are subjected to flood irradiation in the aqueous solution of NQMP-TEG (**2c**, Figure 4). This procedure makes previously unexposed areas highly hydrophilic and significantly reduces protein binding. Longer PEG units attached to NQMP **2** can further increase this effect.

To test the efficiency of the photo-PEGylation procedure, four commercial epoxy-coated glass slides were treated with 2-(2-(vinyloxy)ethoxy)ethanamine under same conditions (Scheme 3). The resulting vinyloxy-derivatized slides were spotted with 2 μL droplets of 0.1 mM aqueous solution of NQMP-TEG-biotin (**2b**) and irradiated for 3 min with 300 nm fluorescent UV lamps. One of these slides was then immersed in 0.1 mM aqueous solution of NQMP-TEG (**2c**) and irradiated for additional 3 min. After FITC-avidin labeling, the slide was rinsed with water and incubated in PBS solution for 1 h. The resulting fluorescent image of this slide shows high contrast between biotinilated and biotin-free areas (Figure 4 D). Three other slides were treated with FITC-Avidin, rinsed, and incubated in PBS solution for 1, 6, and 16 h, respectively (Figure 4A–C). The 1- and 6-h long washing are clearly insufficient to remove nonspecifically absorbed avidin from the slide. Only 16 h washing procedure produces contrast approaching that of photo-PEGylated slide. These experiments show that flood irradiation of biotin-patterned vinyloxy-slide does not decrease the amount of immobilized biotin molecules but drastically reduces nonspecific binding of avidin to the slide.

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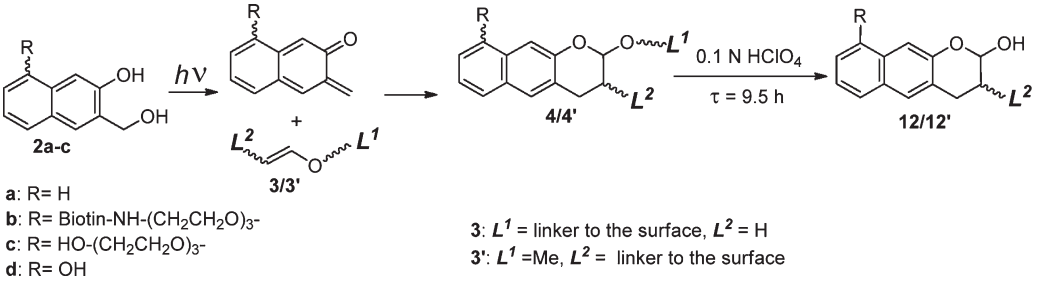
Scheme 4



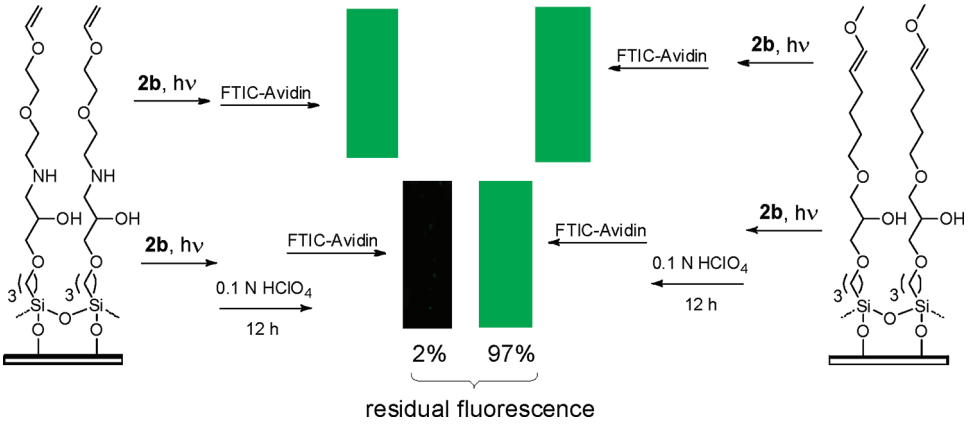


Figure 5. Hydrolytic stability of photochemically biotinylated slides.

Derivatization procedures discussed above produce glass slides with vinyl ether moieties attached to the surface via the vinylic oxygen atom (L^1, Scheme 4). 2-Alkoxy substituent (O-L^1) in benzo[g]chroman **4**, which is formed in the photoclick reaction, is acid labile and can be hydrolyzed off at pH 1 (Scheme 4).[21]

This hydrolytic liability of the linker **4** permits complete removal of the previously immobilized molecules from the glass surface. We have employed acid cleavage procedure to evaluate the efficiency of the photoclick immobilization. For this purpose slides of known epoxide group density (VWR # 16001−030, density = 2×10^{13} molecules per mm^2, surface area =1875 mm^2) were functionalized with vinyl ether groups and subsequently irradiated with 300 nm lamps for 10 min in 0.1 mM aqueous solution of NQMP **2a**. The resulting slides that contained benzochroman **4a** on the surface were incubated in 0.1N perchloric acid solution overnight to induce the release of 2-hydroxybenzochroman **12a** (Scheme 4). The concentration of **12a** in the supernatant solution was determined from its absorbance at 281 nm.[24] The surface density was calculated from triplicate measurements to be $(1.40 \pm 0.06) \times 10^{13}$ of **4a** molecules per mm^2. In other words, all three steps of the process, i.e., vinyl ether functionalization of epoxy slides, photoclick derivatization, and acid-catalyzed release of the substrate, are very efficient proceeding with at least 70% overall yield.

To evaluate the yield of the acid-induced detachment procedure, vinyl ether-derivatized slides were photobiotinilated by irradiation in NQMP **2b** solution. One set of the resulting slides was then incubated overnight in 0.1 M perchloric acid. The acid-treated and the control slides were stained with FITC-avidin, washed in PBS solution overnight, and scanned using Typhoon imager (Figure 5). The fluorescent intensity of the acid-treated slide was less than 2% of the second one, indicating almost quantitative loss of biotin molecules from the surface.

Photo-Diels−Alder click reaction can also be employed in applications that require hydrolytically stable linker between a surface and immobilized substrate. In this case, vinyl ether groups should be attached to the surface via β-carbon atom rather than ether linkage (e.g., **3′** Vs **3** in Scheme 4). We have prepared **3′**-derivatized surfaces by treating the epoxide glass slides with 6-methoxyhex-5-en-1-ol in methylene chloride in the presence of catalytic amount of anhydrous p-toluenesulfonic acid. These slides were then irradiated in an aqueous solution of NQMP **2b** to yield biotinylated slides. One set of these slides was stained with FITC-avidin after irradiation; the second set was incubated in 0.1 M perchloric acid solution overnight before staining. As clearly seen from the Figure 5, incubation in acidic solution did not significantly affect the fluorescent intensity of photobiotinylated slides, indicating that density of biotin groups on the surface remained the same. This observation underscores the stability of linker **4b′** (Scheme 4) to acid-catalyzed hydrolysis.

■ CONCLUSIONS

The photoclick immobilization strategy described in this work is based on the fast and efficient hetero-Diels−Alder cycloaddition of photochemically generated *o*-naphthoquinone methides (*o*NQMs) to immobilized vinyl ether moieties. Since vinyloxy-derivatization of the surface is achieved via a simple and efficient process and a wide variety of substrates can be attached to naphthoquinone methide precursors, 3-(hydroxymethyl)-2-naphthols (NQMP), this method offers a new platform for

light-directed surface functionalization. Photo-Diels–Alder patterning approach is orthogonal to other derivatization techniques and can be used in conjunction with well-developed acetylene–azide click chemistry. The high rate of *o*NQMs cycloaddition to vinyl ether moieties makes this photochemical derivatization strategy very efficient. The unreacted *o*NQMs are quenched with water to regenerate NQMP. The competition between hydration and cycloaddition makes *o*NQMs very selective: only vinyl ethers are reactive enough to outcompete addition of water to the substrate. We also describe a new photopatterning paradigm, in which photochemically inert surface is selectively irradiated in a solution of a photoactive component. The short lifetime of photogenerated reactive species (*o*NQM) limits their migration from the site of irradiation and permits high spatial resolution of the patterning process. The photoclick immobilization technique comprising *o*-napthoquinone methide precursor and vinyl ether can be tailored to produce permanent or a hydrolytically labile linkage.

■ ASSOCIATED CONTENT

Supporting Information. Experimental procedures, preparation and NMR spectra of newely synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
vpopik@uga.edu

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