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Photoreactions on Surfaces

(Bio)Molecular Surface Patterning by Phototriggered Oxime Ligation**

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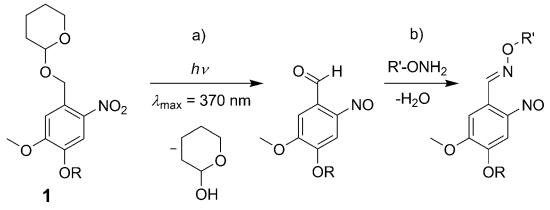
Photolabile moieties are widely employed in organic chemistry and beyond as a powerful tool for breaking bonds smoothly without the need for any additional reagents.[1] Such orthogonal and mild photolytic cleavage is particularly attractive for solid-supported organic synthesis,[2] in the field of combinatorial library screening,[3] and for the tracking of molecular dynamics in biological systems.[4] Of the myriad of photocleavable protecting groups which have been studied, the *o*-nitrobenzyl group is certainly the predominant one since it enables the caging of a wide range of functionalities, such as carboxy,[5] amine,[6] hydroxy,[7] and thiol.[8] In addition, the use of light as a trigger provides a straightforward means to obtain spatial and temporal control over a desired molecular cleavage,[9] which has found significant attention in constructing patterns of multiple cell lines,[10] the production of 3D structured materials for tissue scaffolds,[11] and the photo-caging of active compounds[12] for instance. Furthermore, the precise positioning of *o*-nitrobenzyl groups in polymer chains has allowed for the controlled alteration of polymer properties upon light stimulus, as demonstrated by the fabrication of cytocompatible 3D hydrogels that can photodegrade[13] or photorelease peptides,[14] amphiphilic dendrimer-based photodestructible micelles,[15] or photocleavable block copolymers to produce functionalized nanoporous films,[16] to name but a few.[17]

The underlying mechanism for the photocleavage of *o*-nitrobenzyl derivatives is well studied[18] and typically leads to the release of the protecting group in the form of a nitrosobenzaldehyde derivative as a byproduct.[19] The idea presented herein, however, is to take advantage of this photoreleased aldehyde moiety for oxime formation (see Scheme 1).[20] In other words, to consider the usual byproduct as the actual product. As alluded to above, the use of light would also provide a facile means to confer spatial and temporal control to the increasingly employed oxime-based click chemistry. To date, only Maynard and co-workers have reported molecular patterns employing oxime ligation, however the reaction requires rather harsh conditions, such as electron-beam or photoacid generator-based photolithographies.[21] Interestingly, our method presented herein proceeds at less-energetic wavelengths (UVA) than light-triggered strategies recently reported by us and others, which are based on the nitrile imine-ene 1,3-dipolar cycloaddition (UVC)[22] or on the Diels–Alder cycloaddition of *o*-quinodimethanes[23] or 3-(hydroxymethyl)naphthalene-2-ol derivatives[24] (UVB). Altogether, these different techniques form a versatile toolbox for photopatterning in different contexts, yet all with high efficiency under ambient conditions. The current approach proceeds in two steps: a) a fast and mild photodeprotection and b) the subsequent oxime ligation reaction (see Scheme 1). The 2-[(4,5-dimethoxy-2-nitrobenzyl)oxy]tetrahydro-2*H*-pyranyl (NOTP) scaffold in compound **1** was selected as a novel highly reactive photocleavable moiety based on its overall kinetics—photocleavage quantum yield of *o*-nitroveratryl ethers is one order of magnitude higher than that of



Scheme 1. Photoinduced cleavage of a 2-[(4,5-dimethoxy-2-nitrobenzyl)oxy]tetrahydro-2*H*-pyranyl (NOTP) derivative and subsequent oxime ligation with hydroxylamine derivatives. R = C_3H_6COOH.

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most other *o*-nitro-veratryl derivatives[4]—as well as for the ease of incorporation of the (tetrahydropyran-2-yl)oxy group through dihydropyran (see the Supporting Information for full synthetic procedure).

Initial model reactions were performed in solution on a poly(ethylene glycol) methyl ether (PEG) functionalized with **1**, a system amenable to mass spectrometry, a method which allows the detection of potential side-product formation with much higher sensitivity and specificity than ^{1}H NMR spectroscopy.[25] A low-cost compact fluorescent lamp ($\lambda_{max} = 370$ nm, 14 mW cm^{-2}, 18 W) was employed as the UVA source for the photodeprotection. The outcome of the deprotection is shown in Figure 1, which depicts the mass spectrum of the fully photodeprotected nitrosobenzaldehyde-capped PEG **3**. Complete cleavage upon mild irradiation was typically achieved within only 3 min at ambient temperature (see also Supporting Information Figure S29 for UV spectra). Subsequent overnight reaction of **3** with hydroxylamine hydrochloride resulted in the desired aldoxime **4** (Figure 2). The presence of a small quantity of impurities may also be observed, which can all be assigned to the multiple side-reactions undergone by the nitroso moiety,[26] for example, dimerization yielding **5**, two- and four-electron reduction leading to **6** and **8**, or subsequent condensation generating **7**. Owing to the undesirable nitroso-centered reactions this strategy is not suitable for polymer–polymer conjugation in solution, as such reactions are subject to a stringent set of click requirements.[27] However, it is noteworthy that all the products derived from the reaction between **3** and hydroxylamine contain the desired oxime bond. Consequently, when the reaction sequence is performed on a surface, no influence on the grafting density or the spatial control is to be expected. These findings, as well as the light-based nature of the conjugation technique, prompted us to translate it to the spatially constrained grafting of molecules onto surfaces to produce molecular patterns.

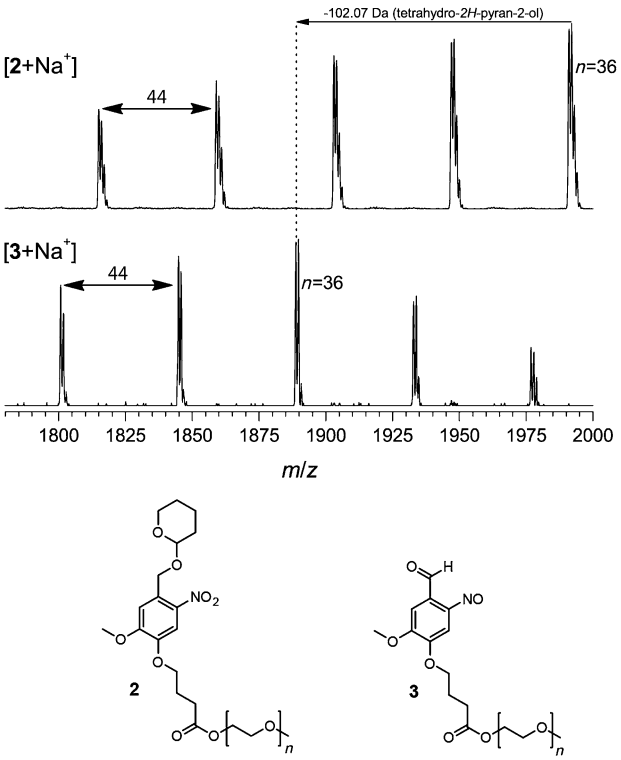


Figure 1. ESI-MS spectra of NOTP-capped poly(ethylene glycol) methyl ether before (top) and after irradiation (middle; $\lambda_{max} = 370$ nm, 14 mW cm^2, 18 W, 3 min). Bottom: Structural formulae for starting compound **2** and photoproduct **3**.

The synthetic route to functionalize silicon surfaces is straightforward. A NOTP-functionalized silane was prepared (Scheme 2), dissolved in anhydrous toluene, and used to treat activated silicon wafers. Upon successful silanization—as demonstrated by X-ray photoelectron spectroscopy (XPS, see

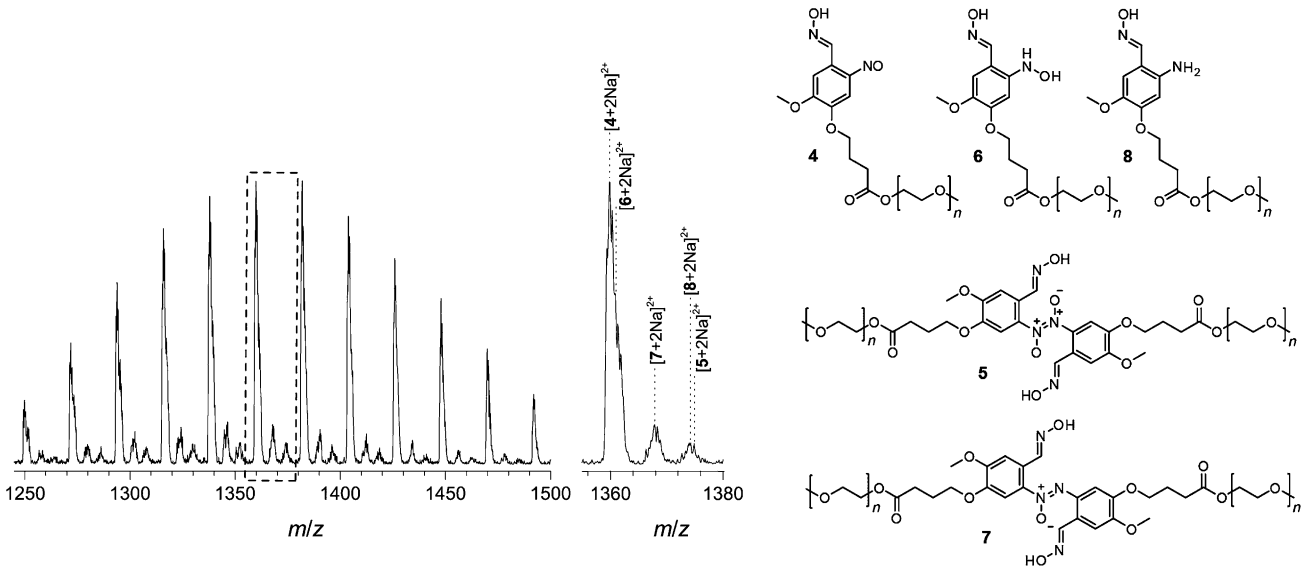
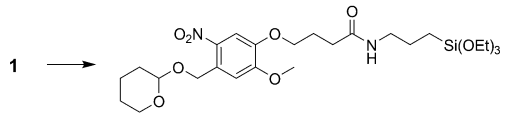


Figure 2. ESI-MS spectrum of nitrosobenzaldehyde-capped poly(ethylene glycol) methyl ether **3** after reaction with hydroxylamine hydrochloride to form **4**. Species **5**, **6**+**8**, and **7** can be assigned to dimerization, reduction, and condensation of the nitroso moiety, respectively. See the Supporting Information for details.



Scheme 2. Synthesis of the NOTP-functionalized silane. Reagents: ethyl chloroformate, triethylamine, 3-(triethoxysilyl)propan-1-amine, THF.

Figure S35)—the photopatterning was achieved by irradiation of the silicon wafer covered with a shadow mask for 3 min (2.5 J cm^{-2} UV dose, lower or comparable to that of the most efficient soft photopatterning techniques[28]). Subsequently, the mask was removed and the silicon wafer was immersed in a solution of *O*-[(perfluorophenyl)methyl] hydroxylamine hydrochloride, which was utilized as a molecular marker to spatially map the locally constrained surface grafting (Figure 3a). Analysis of the photopatterning was by time-of-flight

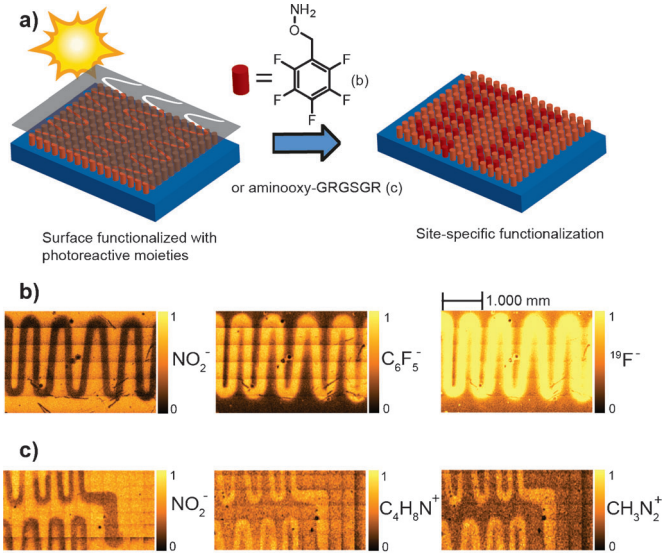


Figure 3. a) Schematic representation of the photodeprotection of Si wafers (blue) functionalized with NOTP (orange) using a shadow mask (gray), and subsequent patterning with *O*-[(perfluorophenyl)-methyl)] hydroxylamine hydrochloride. b) ToF-SIMS images after photodeprotection (left) and oxime ligation with the fluoro marker (center and right). c) ToF-SIMS images after photodeprotection and oxime ligation with aminooxy-GRGSGR (structure included in the Supporting Information).

secondary ion mass spectrometry (ToF-SIMS), which is a powerful and highly sensitive technique for the spatially resolved analysis of molecular patterns on solid substrates.[29] In contrast to traditional fluorescence imaging, ToF-SIMS data also provides detailed information on the chemical composition, essential for analysis of non-fluorescent (bio)-molecules.

A ToF-SIMS composition analysis of the nitro and fluorine contents on the surface after irradiation and functionalization readily reproduced the shadow mask structures with a good spatial resolution between irradiated and non-irradiated areas. Indeed, only the non-irradiated zone showed the presence of nitrite (NO_2^-) and tetrahydro-2*H*-pyranyl ($C_5H_9O^+$) ions (Figure S37)—fragments initially present in the protected molecule—while only the irradiated part exhibited fluorine functionalization, as demonstrated by the detection of F^- and $C_6F_5^-$ ions, after immersion of the wafer in the solution of *O*-[(perfluorophenyl)methyl] hydroxylamine hydrochloride (Figure 3b).

To also demonstrate the feasibility of a covalent and site-specific attachment of biomolecules by this phototriggered approach, we irradiated a freshly prepared NOTP-functionalized wafer and treated it with a solution of (2-aminooxy)acetamido Gly-Arg-Gly-Ser-Gly-Arg peptide (GRGSGR). ToF-SIMS again provided evidence that the peptide was immobilized in a pattern corresponding to the mask features (Figure 3c). In that case, composition analysis was based on the presence of $CH_3N_2^+$ and $C_4H_8N^+$ ions, characteristic secondary ions for arginine-containing peptides.[30]

In summary, we have presented the highly efficient phototriggered deprotection of a novel *o*-nitrobenzyl acetal at 370 nm. The strategy allows not only the quantitative and extremely rapid photorelease of aldehyde functionalities but also for the first time the spatial control over the click-type oxime ligation using mild conditions. Indeed, we translated the two-step procedure to the fabrication of patterned silicon surfaces and confirmed the site-specific attachment of aminooxy-functionalized (bio)molecules, that is, a small marker molecule and a peptide, by ToF-SIMS. We envisage that the introduced technique can be extended to the production of functional patterned substrates to study/control cell behavior. However, the potentially cell-toxic nitroso group should be first neutralized, for example, with glutathione.[31]

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