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A 'photorelease, catch and photorelease' strategy for bioconjugation utilizing a *p*-hydroxyphenacyl group†

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A bioorthogonal 'catch and photorelease' strategy, which combines alkyne–azide cycloaddition between *p*-hydroxyphenacyl azide and alkyne derivatives to form a 1,2,3-triazole adduct and subsequent photochemical release of the triazole moiety *via* a photo-Favorskii rearrangement, is introduced. The first step can also involve photorelease of a strained alkyne and its Cu-free click reaction with azide.

Bioconjugation is a strategy to form covalent links between two molecules of biological interest,[1] which is commonly used in nucleic acid research,[2] pharmaceutical chemistry[3] or microbiology.[4] While the first procedures used non-specific approaches, such as ionic interactions with negatively charged DNA[5] or reactions of nucleophilic amines,[6] carboxylates[7] and thiols[8] with electrophiles, modern methods rely on targeted functionalization, such as biotin–streptavidin conjugates,[9] Staudinger ligation[10] and click reactions.[4]

Many bioconjugation strategies are based on bioorthogonal reactions which allow for chemical transformations of molecules without the need to protect reactive functional groups. They are used for fluorescent labeling,[11,12] cell detection[13] and many other applications.[14] The most common types of bioorthogonal click reactions are cycloadditions, such as Cu(I)-catalyzed alkyne–azide 1,3-dipolar cycloadditions (CuAAC) or Cu-free click reactions of azides or nitrones with strained alkynes, and inverse electron-demand Diels–Alder reaction of 1,2,4,5-tetrazines with strained alkynes or alkenes.[15–17] Photochemically triggered bioorthogonal reactions, which enable spatial and temporal control over the bioconjugation and can be utilized in site-specific functionalizations, are based on photolabile diazirines,[18] tetrazoles,[19–21] and cyclopropenones.[22]

The concept of 'catch and release', involving selective binding of a species from the reaction media, followed by its controlled liberation, has been used, for example, in combinatorial and solid-phase synthesis of complex natural products[23] and biologically active molecules,[24] DNA nanomachine[25] applications, supramolecular host–guest chemistry[26] or resin purification methods.[27] The majority of these methods are based on non-photochemical processes. Nevertheless, involvement of a photochemical step (release) in this strategy has been demonstrated on photocleavable polymers,[28] dendrimers,[29] and liposomes.[30]

A reversible version of click reactions would be highly demanded in the regulation and reversible on–off switching of biological processes, advanced surface modifications, or solid-phase synthesis. However, the chemical stability of the products of click reactions makes the reverse process very difficult. Several attempts for reversible bioorthogonal reactions have been reported. Aside from the retracted scientific report,[31] only a few 'catch and release' strategies suitable for biological applications have been reported. Catch and release DNA decoys are rare examples of non-natural DNA probes that capture and dissociate from DNA-binding proteins upon irradiation of the 4-nitroindole linker.[32] Such a strategy has been demonstrated earlier by Porter and coworkers who developed a reagent containing azido and biotin groups separated by a photocleavable linker based on a benzoin photoremovable protecting group for isolation of protein adducts of lipid-derived electrophiles using streptavidin beads.[33] In addition, Popik and coworkers have recently developed a reversible derivatization technique for peptides and proteins based on the reaction of photochemically generated 2-napthoquinone-3-methides.[34]

Photoremovable protecting groups (PPGs) allow for the precise spatial and temporal release of biologically active molecules.[35] The 4-hydroxyphenacyl photoremovable (*p*HP) protecting group has been shown to liberate rapidly ($k_{obs} = (7–100) \times 10^8\ s^{-1}$) and efficiently ($\Phi = 0.1–1.0$) a wide range of leaving groups.[36–40]

In this work, we present a 'catch and photorelease' strategy which combines alkyne–azide 1,3-dipolar cycloaddition between *p*-hydroxyphenacyl azide **1** and alkyne **2** derivatives to form a 1,2,3-triazole linkage in adduct **3** in the first ('click' or 'catch') step, and subsequent selective irreversible photochemical

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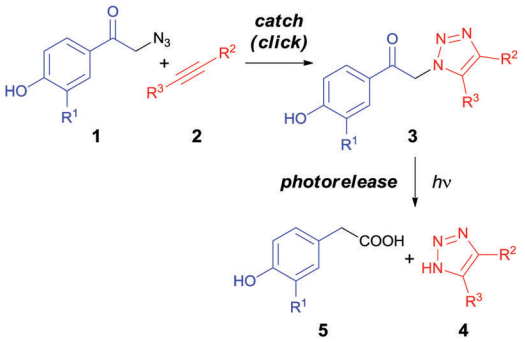


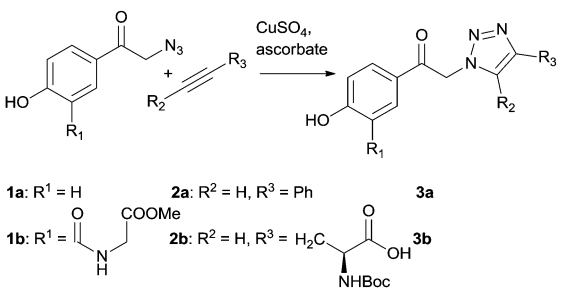
Scheme 1 A "catch and photorelease" strategy.

release of 1-*H*-1,2,3-triazole **4** and 4-hydroxyphenylacetic acid **5** moieties *via* a photo-Favorskii rearrangement[41,42] (Scheme 1). In addition, the first step can involve either a Cu(I)-catalyzed or Cu-free alkyne–azide process; a strained alkyne for the latter approach can be generated from the corresponding cyclopropenone *via* photochemical decarbonylation.[22] We show that these strategies can facilitate efficient bioorthogonal sequential connection and splitting of two (bio)molecules.

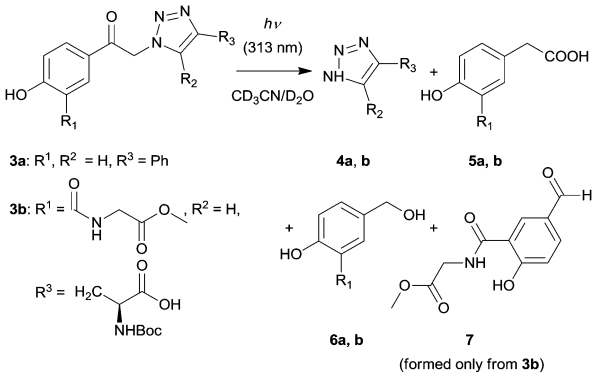
Two *p*HP triazole derivatives **3a** and **b** were synthesized by a CuAAC click ('catch') approach from *p*HP azide (**1a** and **b**) and acetylene (**2a** and **b**) derivatives using $CuSO_4$ and sodium ascorbate in water in 52% and 38% isolated chemical yields, respectively (Scheme 2 and ESI†).[43] The *O*-methylglycine group in the 3-position of **1b** represents a stable amide linkage between the photoactive *p*HP group and a peptide chain.

The second step of this strategy (Scheme 1) involves photochemical release of a triazole moiety from the *p*HP cage. Irradiation of **3a** in CD_3CN/D_2O (1 : 1, v/v) at λ = 313 nm to >99% conversion gave 4-phenyl-1-*H*-1,2,3-triazole (**4a**) as the major photoproduct in quantitative yield and 4-hydroxyphenylacetic acid (**5a**, 80%) and 4-hydroxybenzyl alcohol (**6a**, 20%) as side-products, identified by comparison of their NMR spectra with those reported before[44,45] and using authentic compounds (Scheme 3; Fig. S1, ESI†). Acid **5a** is a characteristic product of the photo-Favorskii rearrangement;[46,47] but benzyl alcohol (such as **6a**) also often accompanies the photolysis of *p*HP derivatives.[41] The major side-photoproducts **5a** and **6a** are water-soluble and have negligible molar absorption coefficients at 313 nm; thus they do not act as internal optical filters.

The necessary role of water in the *p*HP photodegradation mechanism[36,41,48] predetermines this transformation for



Scheme 2 Formation of triazole derivatives in the 'catch' step.



Scheme 3 Release of 1,2,3-triazoles from the *p*HP PPG **3**.

biocompatible aqueous solutions. Acetonitrile in CD_3CN/D_2O (1 : 1, v/v) mixtures was used to increase the solubility of the systems, and it resulted in the formation of considerable amounts of the second byproduct **6**, which would not be formed in pure water.[41]

Compound **3b** represents a moiety that can interlink the photoactive *p*HP group with another molecule *via* a peptide chain. Upon irradiation, this peptide tether, which could bear another molecule or a functional group of interest, remains on the photoproduct(s) formed from the *p*HP chromophore. The *m*-substitution of the *p*HP group by an amido group was chosen because, unlike substituents in the *p*- and *o*-positions,[47] *m*-substituents do not significantly affect the photophysical properties of *p*HP.[49] Exhaustive irradiation of **3b** in CD_3CN/D_2O (1 : 1, v/v) at λ = 313 nm led to the formation of free triazole **4b** in quantitative yield and compounds **5b** (≈25%) and **6b** (≈20%) as the major photo-Favorskii rearrangement products (Fig. S2, ESI†). In addition, *p*-hydroxybenzaldehyde derivative **7** was identified as another side product (Fig. S41, ESI†). Because this compound appeared only after prolonged irradiation, we assume that it is a secondary product formed by **6b** oxidation in the presence of oxygen in air.

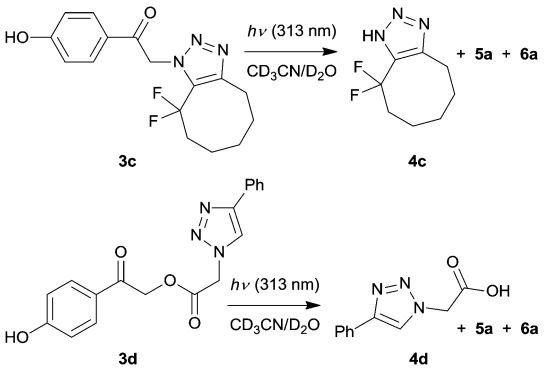
It has been shown that poor leaving groups such as alcohols or amines possessing pK_a values above ≈11 cannot be efficiently released from the *p*HP group.[35,38] For example, phenolate (pK_a ≈ 10) or *p*-cyanophenolate (pK_a ≈ 7.2) is released with a quantum yield of 0.04 and 0.11, respectively.[38] Therefore, because the pK_a value of 1*H*-1,2,3-triazole is 9.4,[50] a rather low release quantum yield from its *p*HP derivative **3a**, $\Phi_{dis} = 9 \times 10^{-4}$ (Table 1), was anticipated.

To increase the quantum yield of photorelease, two modified click adducts **3c** and **3d** were synthesized using a SPAAC (strain-promoted azide–alkyne cycloaddition) and a CuAAC procedure, respectively (Scheme 4 and ESI†). Compound **3c** possesses two electron withdrawing fluorine atoms in the near proximity of the triazole core, which lower its pK_a value and make it a better leaving group. Indeed, the Φ_{dis} value of **3c** was enhanced by a factor of ≈30 compared to that of **3a** (Table 1). Because the triazoles **3a**, **3b** and **3d** were synthesized by CuAAC in the presence of Cu^I ions, which are toxic due to the generation of reactive oxygen species,[51] the non-catalyzed click (SPAAC)

Table 1 Disappearance quantum yields of *p*HP derivatives **3**[a]

*p*HP derivative	Φ_{dis}/%
3a[b]	0.09 ± 0.02
3c[b]	2.9 ± 0.4
3d[b]	83 ± 4
3e[c]	0.39 ± 0.05

[a] Irradiated at λ = (313 ± 1.5) nm (**3a**, **3d**) or (320 ± 10) nm (**3c**, **3e**); 4-hydroxyphenacyl fluoride[39] was used as an actinometer. [b] In a mixture of acetonitrile (10%) and acetate buffer (pH = 5.0). [c] In a mixture of acetonitrile (20%) and acetate buffer (pH = 5.0).

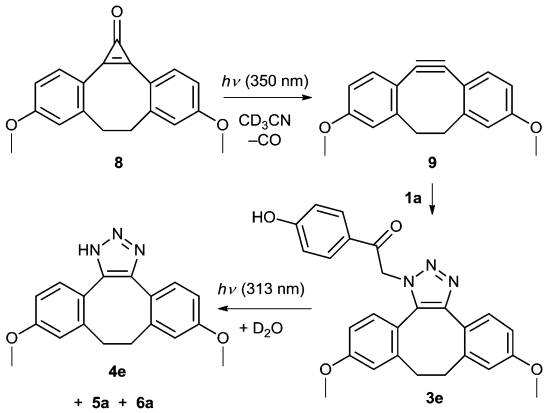


Scheme 4 Photochemistry of **3c** and **d**.

synthesis of the DIFO-derivative[52] **3c** represents a more biocompatible method.

Because controlling the pK_a values of triazoles is structurally limited, we adopted a different approach. The poor leaving ability of alcohols, phenols or amines can be enhanced by connecting them to the chromophore through a carbonate[53,54] or carbamate[55] linker. Here we modified the triazole moiety through a methylcarbonyloxy linker in **3d**, where the leaving group is a carboxylate; thus the pK_a value of its conjugate acid is approximately 4. The Φ_{dis} value of 0.83, which is 3 orders of magnitude higher than that of **3a**, is within the values found for other *p*HP esters (Φ_{dis} = 0.4–0.94)[38] and represents a significant improvement for any future applications. A measure of the photorelease (the uncaging cross section), the product of the quantum yield and the decadic molar absorption coefficient, $\Phi\varepsilon(\lambda_{irr})$,[35] for **3d** at 313 nm (ε_{313} = 2.73 × 10^3 M^{-1} cm^{-1}) is remarkably large (2.27 × 10^3 M^{-1} cm^{-1}).

We further modified the first step of the 'catch and photorelease' strategy by introducing an angle-strained cycloalkyne which allows for efficient Cu-free click chemistry *via* facile 1,3-dipolar cycloaddition (SPAAC).[17,56,57] Such a reactive alkyne can be efficiently photochemically produced *in situ* from its cyclopropenone precursor, which is an elegant solution to spatial and temporal control of the release.[22,58] In this work, cyclopropenone **8** was synthesized by a 2-step procedure in 27% overall yield (Scheme 5 and ESI†) using a LiNK metalation-coupling procedure.[59] This is a significant improvement when compared to a previously reported 4-step synthesis[60] of cyclopropenone **8**. It has been shown that this compound photodecarbonylates with a quantum yield of 0.33 to give a strained alkyne **9**, which



Scheme 5 'Release, catch and release' strategy.

can undergo non-photochemical SPAAC reaction with azides.[22] Thus, a solution of **8** and *p*HP azide (**1a**; 1 equiv., $c \approx 5$ mg mL^{-1}) in acetonitrile was irradiated at a wavelength of 350 nm, at which only **8** absorbs (Fig. S6, ESI†). The generated alkyne **9** underwent *in situ* non-photochemical SPAAC reaction with **1a** to form **3e** (quantitative yield determined by ^{1}H NMR; 76% isolated yield). Upon irradiation at 313 nm, this *p*HP derivative was converted quantitatively into triazole **4e** and *p*-hydroxybenzyl derivatives **5a** and **6a**, which could bear a peptide tether to connect the released moiety with a biomolecule, *via* the photo-Favorskii rearrangement. The disappearance quantum yield of **3e** was higher than that of **3a** because **4e** is a better leaving group compared to **4a**.

Our 'photorelease, catch and photorelease' strategy is a one-pot multistep procedure, which is solely controlled by light, and does not require any other reagents besides water. In addition, both photochemical steps are orthogonal. The *p*HP group does not absorb at a wavelength of 350 nm (Fig. S39, ESI†) used for generation of strained alkyne **9**; thus cycloadduct **3e** remains unreacted in the solution during the first irradiation step. The *p*HP chromophore transformations rely on irradiation at wavelengths in the UV region ($< \approx 320$ nm), which may limit their bioapplications. However, 2-photon excitation could overcome this issue, as was recently successfully demonstrated by Givens and coworkers on *p*HP-caged diethyl phosphate and ATP using a 550 nm laser.[40]

In conclusion, we synthesized a series of substituted *p*HP azides and alkynes which can be interlinked using an alkyne–azide 1,3-dipolar cycloaddition protocol and subsequently be released upon irradiation with UV-light *via* a photo-Favorskii rearrangement. We show that the reactions can be accomplished in aqueous media favorable for biological applications and that the linker can be used to connect/disconnect two (bio)molecules. A rational design of the leaving group (triazole) structure led to the development of a linker that cleaves upon irradiation with near-unity quantum yield. The second generation of this strategy encompasses chromatically orthogonal photoproduction of a strained alkyne which allows for Cu-free click chemistry. We propose that the systems could be used in bioconjugation or drug-delivery applications.

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