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Light-Induced Hetero-Diels–Alder Cycloaddition: A Facile and Selective Photoclick Reaction

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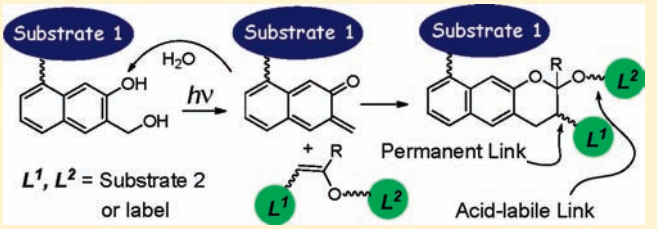
Supporting Information

ABSTRACT: 2-Napthoquinone-3-methides (*o*NQMs) generated by efficient photodehydration (Φ = 0.2) of 3-(hydroxymethyl)-2-naphthol undergo facile hetero-Diels–Alder addition ($k_{D\text{-}A} \sim 4 \times 10^4\ M^{-1}\ s^{-1}$) to electron-rich polarized olefins in an aqueous solution. The resulting photostable benzo[g]chromans are produced in high to quantitative yield. The unreacted *o*NQM is rapidly hydrated ($k_{H2O} \sim 145\ s^{-1}$) to regenerate the starting diol. This competition between hydration and cycloaddition makes *o*NQMs highly selective, since only vinyl ethers and enamines are reactive enough to form the Diels–Alder adduct in an aqueous solution; no cycloaddition was observed with other types of alkenes. To achieve photolabeling or photoligation of two substrates, one is derivatized with a vinyl ether moiety, while 3-(hydroxymethyl)-2-naphthol is attached to the other via an appropriate linker. The light-induced Diels–Alder "click" strategy permits the formation of either a permanent or hydrolytically labile linkage. Rapid kinetics of this photoclick reaction ($k = 4 \times 10^4\ M^{-1}\ s^{-1}$) is useful for time-resolved applications. The short lifetime ($\tau \sim 7$ ms in H_2O) of the active form of the photoclick reagent prevents its migration from the site of irradiation, thus, allowing for spatial control of the ligation or labeling.



INTRODUCTION

Connection (or ligation in biochemistry) of two or more substrates or immobilization of various compounds is often achieved with the help of click chemistry. The term "click chemistry" was introduced by K. Barry Sharpless to describe a set of bimolecular reactions that are "modular, wide in scope, high yielding, create only inoffensive by-products, are stereospecific, simple to perform and that require benign or easily-removed solvent".[1] Although meeting all of the above requirements is difficult to achieve, several processes have been identified as coming very close to the ideal click reaction. Among them are 1,3-dipolar and Diels–Alder cycloadditions, nucleophilic ring-openings, nonaldol carbonyl chemistry, and addition to carbon–carbon multiple bonds. Cu(I)-catalyzed versions of the Huisgen acetylene-azide cycloaddition, also known as azide click reaction, became the gold standard of click chemistry and have been applied in fields ranging from materials science[2] to chemical biology[3,4] and drug development.[5] However, copper ions are cytotoxic,[6] can cause degradation of DNA molecules[7] and aggregation of azide-labeled antibodies,[8] as well as induce protein denaturation.[9] In addition, copper complexation hampers functionalization of substrates with chelating agents (e.g., for the introduction of radiolabels).[10] The use of catalysts complicates kinetics of the immobilization process, requires polar solvents, and can alter surface properties.[11] Catalyst-free 1,3-dipolar cycloaddition of azides to cyclooctynes[12] and dibenzocyclooctynes[13] allows for alleviation of these limitations.

Click methods based on a Diels–Alder cycloaddition are gaining popularity due to the fact that this reaction does not require catalysts, can proceed in high yield under physiological conditions, and does not produce any byproducts.[14] The Diels–Alder click reaction has found applications in materials chemistry,[15,16] derivatization of nanoparticles and surfaces with various bioactive molecules,[17] as well as the labeling of oligonucleotides, proteins, and oligosaccharides.[18–20] However, Diels–Alder cycloaddition reactions are often slow and require either thermal activation[21] or the use of chemical promoters for the *in situ* generation of reactive dienes.[22]

The utility of click reaction-based strategies can be further extended by employing photochemically triggered click reactions, as this approach allows for the spatial control of the process. Several photoclick methods are currently under development, including cycloaddition of alkenes to photochemically generated nitrile imines,[23] as well as photoinitiated thiol–ene[24] and thiol–yne[25] reactions.

Here, we report a novel photoclick platform based on a facile and efficient light-induced hetero-Diels–Alder click reaction. Photochemical dehydration of 3-hydroxy-2-naphthalenemethanol derivatives **2** produces *o*-naphthoquinone methides (*o*NQMs) **1**. The latter undergo facile cycloaddition to vinyl ethers **3** or enamines **4** to produce photostable derivatives of benzochromans **5** and **6** (Scheme 1). Unreacted *o*NQM **1** is rapidly hydrated to regenerate starting material **2**. This method allows for efficient ligation under ambient conditions in aqueous solution and does not require catalysts or promoters. In addition,

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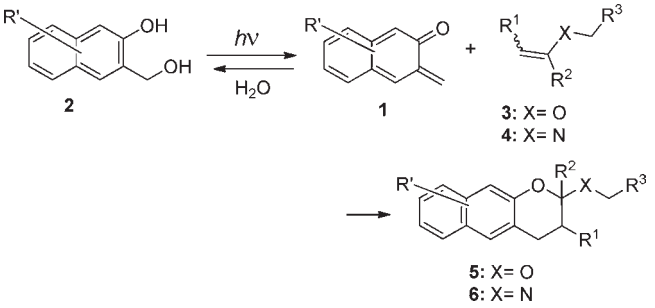
Scheme 1



the fast rate of this reaction permits high spatial and temporal resolution of the ligation.

RESULTS AND DISCUSSION

The 3-hydroxy-2-naphthalenemethanol chromophore (**2**) has two major absorption bands in UV region above 210 nm: at λ_{max} = 275 nm (log ε = 4.06) and at λ_{max} = 324 nm (log ε = 3.70, Figure 1). Introduction of an alkoxy substituent at the 5 position of the chromophore (e.g., **2d**: **R**′ = $(OCH_2CH_2)_3OCH_3$) results in a ca. 10 nm bathochromic shift of both bands. *o*-Naphthoquinone methide precursors **2** also show significant fluorescence with a short lifetime (Φ_{Fl} = 0.230 ± 0.002 with τ_{FL} ~ 7 ns for **2a**[26]). The emission spectrum of **2a** in aqueous solutions contains two major bands at 360 and 423 nm.

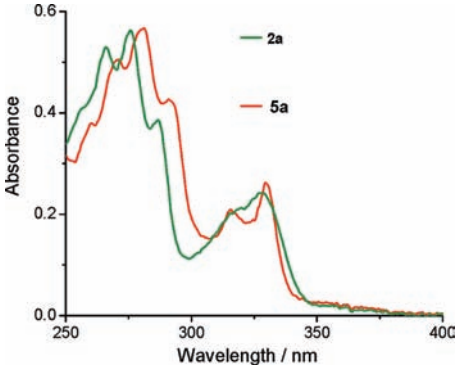


Figure 1. UV-spectra of ca. 10^{-4} M solutions of 3-hydroxy-2-naphthalenemethanol (**2a**, green line) and adduct **5a** (red line) in (1:1) acetonitrile−water.

Irradiation of aqueous solutions of 3-hydroxy-2-naphthalenemethanol derivatives **2** using low pressure Hg lamps (254 nm), as well as 300 or 350 nm fluorescent tubes, results in the efficient dehydration (Φ_{300} = 0.17 ± 0.02 for **2a**)[26] of the substrate and the formation of *o*-naphthoquinone methides (*o*NQM) **1** (Scheme 1). *o*NQMs **1** rapidly react with water (k_{2a} = 145 s^{-1} or 2.61 M^{-1} s^{-1}) to quantitatively regenerate the starting material. In the presence of vinyl ethers **3**, however, *o*NQMs **1** undergo facile Diels−Alder cycloaddition to produce substituted 2-alkoxy-3,4-dihydro-2*H*-naphtho[2,3-b]pyrans **5** in high or quantitative yield (Scheme 2, Table 1). The bimolecular rate of the addition of *o*NQM **1a** to various vinyl ethers in aqueous solutions is (4−6) × 10^4 M^{-1} s^{-1} (Table 2).[26] Vinyl ethers **3** do not react with 3-hydroxy-2-naphthalenemethanol **2a** in aqueous solution, even at elevated temperatures.

Scheme 2

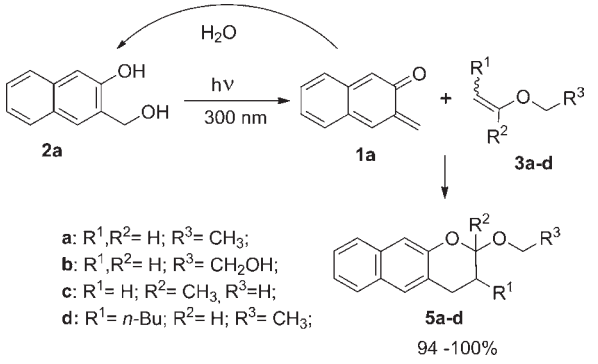


Table 1. Formation of Adducts 5a−d in Photolysis of 2a in the Presence of Vinyl Ethers[a]

olefin	yield **5** (% conversion)
3a	96 ± 2 (>99)
3b	94 ± 2 (>99)
3c	95 ± 3 (>99)
3d	97 ± 2 (>99)

[a] Concentration of **2a** = 1 mM in 50% MeCN and 0.01 N aqueous biphosphate buffer (BR = 1); [vinyl ether] = 1.5 mM; irradiation wavelength = 300 nm.

Table 2. Observed Rates of *o*NQM 1a[a] **Decay of in the Presence of 0.05 M Vinyl Ethers 3a−d**

olefin	observed rate (s^{-1})
3a	3100 ± 121
3b	3050 ± 75
3c	3080 ± 101
3d	3090 ± 66

[a] Generated by 266 nm laser pulse from 0.0001 M solution of **2a** in CH_3CN: 0.01 N biphosphate buffer (BR = 1).

In a typical experiment, a solution of 1 mM of **2a** and 1.5 mM of olefin **3** in a 50% acetonitrile−0.01 N aqueous biphosphate buffer (BR = 1) mixture was irradiated using 300 nm 4 W fluorescent tubes. The yield of adduct **5** was measured using HPLC analysis using pure product as a reference.[27] As illustrated by the data presented in Table 1, the presence of various substituents at the oxygen, α-carbon, and *β*-carbon atoms of vinyl ether **3** has very minimal effect on the yield of the adduct **5**. It is also important to note that nearly quantitative yields of Diels−Alder products **5a−d** were obtained using only a 50% molar excess of vinyl ether. Both 300 and 350 nm light-induced cycloadditions are very clean reactions; no photoproducts other than benzochromans **5** were detected in the reaction mixtures by HPLC. Photolysis using 350 nm light requires longer irradiation time to achieve the same conversion as in 300 nm experiments, apparently due to a lower extinction coefficient of chromophore **2** at this wavelength.

Interestingly, the reactivity of *o*NQM **1a** in the cycloaddition reaction with vinyl ethers is also insensitive to the substitution

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pattern of the latter (Table 2). *o*NQM **1a** was generated by laser flash photolysis of a 0.001 M solution of 3-hydroxy-2-naphthalenemethanol (**2a**) in a 50% acetonitrile−0.01N biphosphate buffer (BR = 1) mixture with 7 ns 266 nm pulses using a Nd:YAG laser. The decay of **1a** in the presence of 0.05 M of vinyl ethers **3a**−**d** was followed using time-resolved UV spectroscopy.[27] The pseudo-first-order rates of the reaction of *o*NQM **1a** with vinyl ethers **3a**−**d** are identical within experimental error.

2-Alkoxybenzochromans **5a**−**d** are photochemically stable and show no decomposition, even after prolonged irradiation at 254, 300, or 350 nm. The hydrolytic stability of **5a** was evaluated by incubating this compound in an aqueous biphosphate buffer (pH = 7.4), a 0.1 M sodium hydroxide solution, as well as a 0.1 M perchloric acid, for 24 h. HPLC analysis of the reaction mixtures indicated no products of hydrolysis and showed no changes in **5a** concentration in aqueous solution at neutral pH and in the presence of 0.1 N sodium hydroxide. At pH = 1, however, **5a** undergoes slow hydrolysis (lifetime $\tau \sim 9.5$ h) to produce benzochroman-2-ol (**5e**, Scheme 3). In other words, light-directed Diels−Alder cycloaddition-based ligation can produce hydrolytically labile (connected via the vinyl ether oxygen atom) or hydrolytically stable linkers (via α- or β-carbon atoms of vinyl ether moiety, Scheme 3).

o-Quinone methides are known to react with various alkenes resulting in the formation of Diels−Alder products.[28] However, when *o*-quinine methides are generated in the presence of water (or an alcohol or thiol), the cycloaddition reaction competes with rehydration that yields back the starting material. This competition can be employed to enhance the selectivity of *o*-quinone methides. To assess the selectivity of *o*NQM addition to alkenes, two set of experiments were carried out. First, *o*NQM **1a** was generated by 300 nm photolysis of 1 mM solutions of 3-(ethoxymethyl)-2-naphthol (**2-Et**) in 50% aqueous acetonitrile in the presence of 0.1 M of the following alkenes: ethyl vinyl ether (**3a**), 2,5-dihydrofuran (**8**), dimethyl maleate (**9**), 1-methylcyclohexene (**10**), methyl acrylate (**11**), and phenyl vinyl ether (**12**). Only in the presence of **3a**, the quantitative formation of the Diels−Alder adduct **5a** was observed, both at low and high conversion (Scheme 4, Table 3). In all other cases, only the product of rehydration, for example, 3-hydroxy-2-naphthalenemethanol (**2a**), was produced. No adducts of **1a** to alkenes **8**−**12** were detected in 50% aqueous acetonitrile (Scheme 4). At low conversion photolysis, **2a** is formed quantitatively, while at higher conversions, the yield of hydration product is somewhat reduced. Since no new photoproducts were detected by HPLC, we believe that the secondary photoproducts are most likely *o*NQM oligomers,[29] which were trapped in the

Scheme 3

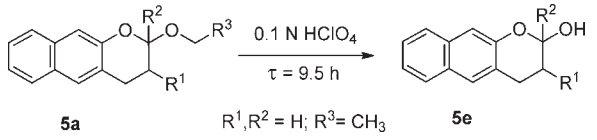

Table 3. Yields of Diels-Alder Adducts and the Hydration Product (2a) Formed in Photolyses of 3-(Ethoxymethyl)-2-naphthol 2-Et in the Presence of Various Olefins 8−12[a]

alkene	yield of **2a** (% conversion)	yield of the Diels−Alder adduct (% conversion)
3a	0 (15)	97 ± 2 (15)
	0 (>99)	96 ± 2 (>99)
3a + **8**−**12**	0 (>99)	97 ± 2 (>99)
8	97 ± 2 (14)	0 (14)
	71 ± 3 (89)	0 (89)
9	93 ± 2 (14)	0 (14)
	69 ± 3 (87)	0 (87)
10	94 ± 2 (14)	0 (14)
	73 ± 2 (90)	0 (89)
11	96 ± 2 (14)	0 (14)
	71 ± 2 (92)	0 (89)
12	95 ± 2 (14)	0 (14)
	71 ± 2 (85)	0 (89)

[a] [Olefin] = 0.1 M; [**2-Et**] = 1 mM. Irradiation wavelength = 300 nm.

Scheme 4

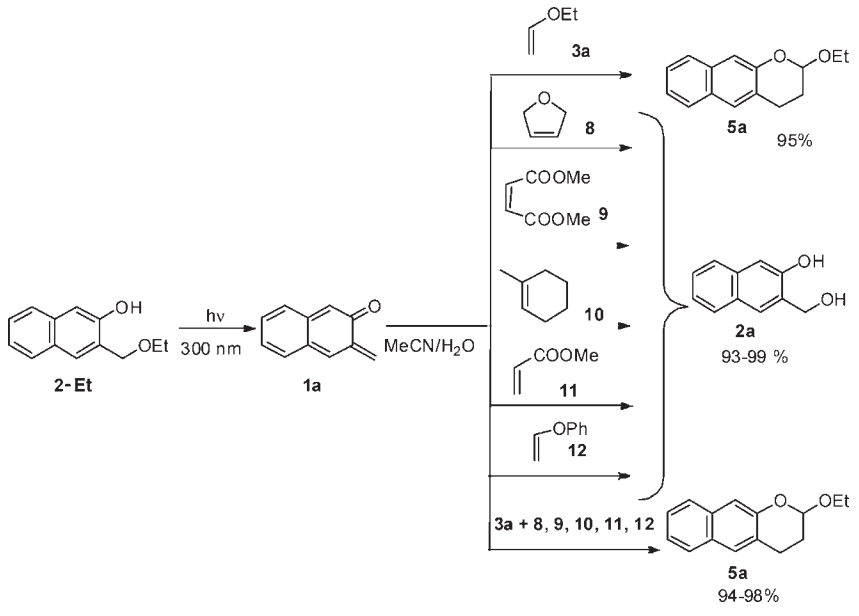

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Table 4. Rate of Decay of NQM 1a in the Presence of Alkenes 3a, and 8−11[a]

alkene	observed rate (s^{-1})
No alkene	119 ± 6
3a	2703 ± 55
8	125 ± 9
9	118 ± 4
10	123 ± 7
11	121 ± 8

[a] Solution in 50% MeCN−0.01 N aqueous phosphate buffer mixture. [alkene] = 0.04 M.

HPLC column. These experiments indicate that while aliphatic vinyl ethers efficiently add to *o*NQMs in an aqueous solution, hydration of **1a** to **2a** outcompetes cycloaddition to other alkenes. It is interesting to note that aromatic vinyl ethers, such as **12**, show substantially lower reactivity in this hetero-Diels−Alder reaction than aliphatic vinyl ethers.

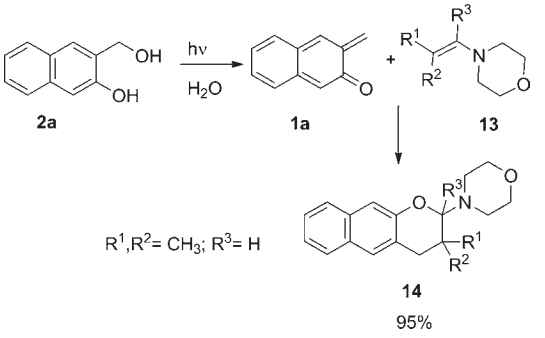
Next, we employed a competitive reaction to directly compare the reactivity of *o*NQMs toward various dienophiles. A 1 mM solution of 3-(ethoxymethyl)-2-naphthol (**2-Et**) in 50% aqueous acetonitrile was irradiated in the presence 0.1 M of ethyl vinyl ether (**3a**) and equal amounts (0.1 M each) of olefins **8**−**12**. HPLC analysis of the reaction mixture showed the clean formation of only one product, vinyl ether adduct **5a**, which was produced in excellent yield (Scheme 4, Table 3). While *o*NQMs only form Diels−Alder adduct with electron rich polarized alkenes in aqueous solutions, the hypothetical possibility exists that *o*NQM adducts of alkenes **8**−**12** are rapidly formed but then undergo facile photochemical or dark decomposition to **1a** or hydrolysis to **2a**. To explore the feasibility of such a process, we have studied the quenching of transient **1a** with various alkenes. *o*NQM **1a** was generated by laser flash photolysis of 3-(hydroxymethyl)-2-naphthol **2a** in 50% aqueous acetonitrile in the presence of 0.04 M of olefins **3a**, and **8**−**11** [Laser flash photolytic experiments using phenyl vinyl ether (**12**) were not performed due to significant extinction coefficient of this compound at the excitation wavelength (266 nm).] The disappearance of **1a** was followed using kinetic spectrometry.[27] In the presence of **3a**, the rate of **1a** decay was significantly enhanced (Table 4). In all other cases, the rate of *o*NQM decay was equal to the rate determined in neat water. This observation clearly indicates that only cycloaddition of *o*NQMs to vinyl ethers is fast enough to outcompete hydration. In the case of nonpolarized olefins and electron poor polarized olefins, the hydration reaction is the only reaction observed.

To explore the reactivity of *o*NQMs toward other important families of electron-rich polarized alkenes, that is, enamines, we irradiated 3-(hydroxymethyl)-2-naphthol (**2a**) in the presence of *N*-(1-isobutenyl)morpholine (**13**). Photochemically generated *o*NQM **1a** rapidly adds to enamine **13**, resulting in quantitative formation of 2-morpholinobenzochroman **14** (Scheme 5).

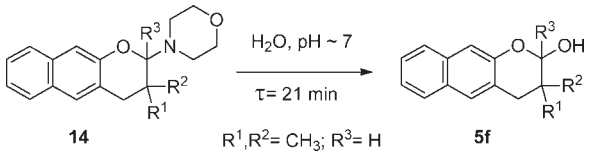
In aqueous solutions, adduct **14** undergoes rapid hydrolytic cleavage of the amino substituent to produce 2-hydroxybenzochroman (**5f**, Scheme 6). The lifetime of **14** in aqueous solution at pH ∼ 7 is $\tau = 21.2 \pm 0.6$ min. Thus, enamines can be also employed in the photoinduced Diels−Alder click ligation if the substrate or label is attached to the α- or *β*-carbon atoms of the enamine.

Reactive *o*NQMs **1** can be efficiently intercepted by good nucleophiles, such as the azide ion and thiols. The bimolecular

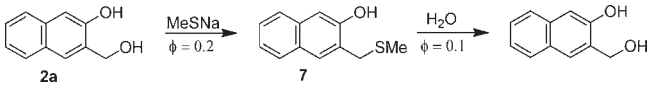
Scheme 5



Scheme 6



Scheme 7



rate of the reaction of *o*NQM **1a** with thioethanolamine (k_{SH} = 2.2×10^5 M^{-1} s^{-1}) is about 5 times faster than the cycloaddition reaction ($k = 4 \times 10^4$ M^{-1} s^{-1}) and the azide ion ($k_{N3} = 2.0 \times 10^4$ M^{-1} s^{-1}) reactivity with *o*NQM is slightly slower. While the azide ion is uncommon in biological systems, concentration of endogenous thiols (e.g., glutathione) can achieve millimolar concentrations. Therefore, thiol quenching of the *o*NQMs **1** can potentially reduce the efficiency of photo-Diels−Alder-based click ligation in biological systems. To explore the properties of *o*NQM−thiol adducts, we prepared thioether **7** by irradiation of **2a** in the presence of sodium salt of methylthiol (Scheme 7). The quantum efficiency of this reaction (Φ = 0.2) is similar to that of the photo-Diels−Alder cycloaddition, and **7** is produced quantitatively. While thioether **7** is hydrolytically stable in both acidic and basic media, irradiation at 300 or 350 nm regenerates *o*NQM **1a** with Φ = 0.1 (Scheme 7). In other words, while thiols are efficient quenchers of *o*NQMs, the resulting thioethers also serve as photoprecursors to reactive quinone methides. To test the ability of *o*NQM to form adduct with vinyl ether in the presence of thiols, we compared results of irradiation of 0.1 mM aqueous solution of **2a** containing 0.15 mM of vinyl ether **3a** with or without the presence of methyl thiol (0.15 mM). In the absence of thiol, **2a** was quantitatively converted into the Diels−Alder adduct **5a** after 20 min of irradiation at 300 nm, whereas in the presence thiol, the Diels−Alder adduct **5a** and thioether **7** were formed in the 3:1 ratio of after 20 min. However, after additional 10 min of irradiation, only **5a** was observed in the photolysate. HPLC analysis confirms quantitative formation of the Diels−Alder product in the latter case. These results indicate that presence of thiols does not prevent the formation of the Diels−Alder adduct but require somewhat longer irradiation.

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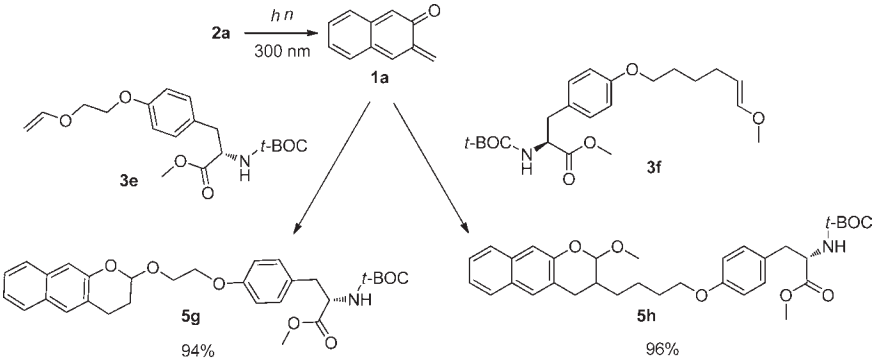
Scheme 8



Another concern for the application of Diels–Alder photoclick labeling in live organisms is the potential cytotoxicity of *o*NQMs **1** and/or their precursors **2**. In fact, many *o*-quinone methide-producing compounds are known to be cytotoxic, mostly due to the ability of *o*-quinone methides to alkylate DNA bases.[30] 3-Hydroxy-2-naphthalenemethanol derivatives (**2**), on the other hand, only form *o*NQMs photochemically because of a poor leaving group in the benzylic position. The cytotoxicity of **2d** was assessed by incubating Jurkat cells in the presence of this compound in the dark and under 350 nm irradiation. MTT assay showed the same viability of treated and control cells.[27]

To demonstrate the utility of the photoinduced hetero-Diels-alder reaction in the ligation experiments, we have prepared tyrosine-vinyl ethers conjugates **3e** and **3f**.[27] In the first one (**3e**), the payload is attached to vinyl ether moiety via the oxygen atom (Scheme 8). Irradiation of *o*NQM precursor **2a** with 300 nm light in the presence of **3e** resulted in the rapid formation of adduct **5g** in 94% isolated yield (Table 5).

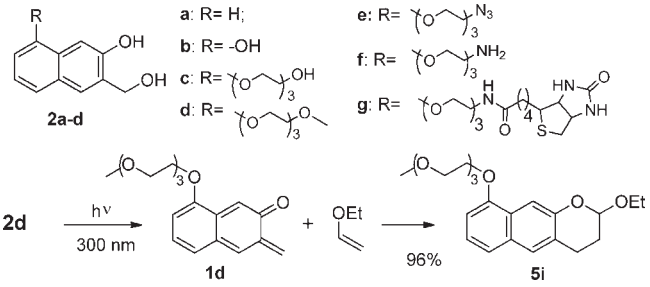
Table 5. Yields of Cycloaddition Products upon Photolysis of *o*NQM Precursors 2a and 2d in the Presence of Various Vinyl Ethers[a]

alkene	*o*NQM precursor	benzochroman	yield (% conversion)
3e	**2a**	**5g**	94 ± 4 (>99)
3f	**2a**	**5h**	96 ± 2 (>99)
3a	**2b**	**5i**	96 ± 2 (>99)

[a] In 50% MeCN–0.01 N phosphate buffer mixture. [alkene] = 1.5 mM; [oNQM precursor] = 1 mM. Irradiation at 300 nm.

The link between the payload and benzochroman moiety in **5g** is hydrolytically labile and can be cleaved by incubating the adduct (**5g**) in an acidic solution. For the applications that require permanent ligation, the payload can be attached to a vinyl ether moiety through either the α- or *β*-carbon atoms. Thus, we prepared methyl vinyl ether derivative **3f**, where a tyrosine unit is attached to *β*-carbon of the vinyl ether through a short linker (Scheme 8). Irradiation of 50% aqueous acetonitrile solution of 3-(hydroxymethyl)-2-naphthol (**2a**) at 300 nm in the presence of 1.5 equiv of vinyl ether **3f** resulted in the quantitative formation of ligation product **5h** (Table 5). The resulting linkage between the benzochroman and tyrosine is stable in both acidic and basic solutions. Cycloadducts **5g** and **5h** are stable under 300 or 350 nm irradiation.

Scheme 9



The label or second component for ligation has to be attached to the naphthalene ring of the *o*NQM precursor **2** via an appropriate linker (Scheme 1). We have demonstrated that various functional groups can be attached via a triethyleneglycol (TEG)-linker to 3,8-dihydroxy-2-naphthalenemethanol (**2b**–**g**, Scheme 9).[27] Introduction of a thriethylene glycol substituent in 8-position of a *o*NQM precursor does not affect its photochemistry. Thus, irradiation of a solution of the triethylene glycol ether of 3,8-dihydroxy-2-naphthalenemethanol (**2d**) in 50% aqueous acetonitrile in the presence of ethyl vinyl ether (**3a**) quantitatively produces adduct **5i** (Table 5). Product **5i** was isolated in 96% yield and fully characterized. Since azide groups are photoreactive at shorter wavelengths, we have investigated the photostabilty of the oNQM precursor **2e** that contains azido functionality at 300 nm. HPLC analysis clearly indicates that there is no change in the concentration of starting material after 30 min of irradiation at 300 nm in aqueous solution. This control experiment clearly demonstrates that the azide group remains intact at the Diels–Alder photoclick conditions. Such bifunctional linker molecules allow for combining light-directed ligation with conventional azide-acetylene click chemistry.

CONCLUSION

The photochemical Diels–Alder cycloaddition described in this report offers a new platform for light-induced ligation, which has potential to serve as orthogonal click system to the widely employed acetylene-azide click chemistry. Irradiation of 3-(hydroxymethyl)-2-naphthols produces 2-napthoquinone-3-methides (*o*NQMs), which react rapidly with vinyl ethers to produce 2-alkoxybenzochromans. *o*NQM precursors and vinyl

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ethers do not react in the dark. The high photochemical stability of Diels−Alder adducts eliminates secondary photoreactivity. The fast rate of the *o*NQM addition to vinyl ethers coupled with short lifetimes of quinone methides makes this method especially well-suited for applications requiring spatial and temporal resolution. Photochemically generated *o*NQMs are also very selective: in aqueous solution, only electron-rich polarized alkenes produce Diels−Alder adducts. The unreacted *o*NQMs are quenched with water to regenerate starting material. The photo-click ligation technique comprising *o*-napthoquinone methide precursor and vinyl ether can be tailored to produce permanent or a hydrolytically labile linkage. We are currently investigating the utility of photo-Diels−Alder click chemistry for fluorescence labeling of live cells, protein derivatization, and patterned immobilization of biomolecules on various surfaces.

■ ASSOCIATED CONTENT

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

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