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Light Induced Ligation of *o*-Quinodimethanes with Gated Fluorescence Self-Reporting

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ABSTRACT: We introduce a highly efficient photoligation system, affording a pro-fluorescent Diels-Alder product which on demand converts into an intensively fluorescent naphthalene *via* E1-elimination in the presence of catalytic amounts of acid. The Diels-Alder reaction of the photocaged diene (*o*-quinodimethane ether or thioether, *o*-QDMs) with electron deficient alkynes is induced by UV or visible light. In contrast to previously reported ligation techniques directly leading to fluorescent products, the fluorescence is turned on after the photoligation. Thus, the light absorption of the fluorophore does not undermine the photoligation *via* competitive absorption and as a result, photobleaching or side reactions of the fluorophore are not observed. Critically, the gated generation of a fluorescent product allows for fluorometric determination of the conversion. We employ a simple synthesis strategy for heterobifunctional electron deficient alkynes allowing for facile functionalization of payload molecules.

Photocaged dienes based on *o*-methylbenzaldehydes (*o*-MBAs) are frequently used in various applications, such as polymer network formation[1], single chain nanoparticle (SCNP) folding[2], 3D-laser writing[3] and many more, as they offer the attractive feature of spatio-temporal control and serve as indispensable tool in contemporary polymer and supramolecular chemistry.[4-7] A limitation of the ligation of *o*-MBAs and electron deficient enes is the lack of a simple visual feedback that allows for monitoring the reaction progress and selectivity in solution and on surfaces.[8] Many common photoinduced reactions, for instance the nitrile imine tetrazole ene-reaction click reaction (NITEC),[9] or the reaction of photocaged naphtoquinone-3-methides (*o*-MQMs) with vinylethers[10,11] form fluorescent products (pyrazoles, pyrazolines and naphtalenes, respectively). Despite this advantage, the direct formation of reaction products that either absorb in the same region or are red-shifted in their absorption compared to their starting materials, represents a considerable challenge. The competitive absorption of photons by the reaction products, decreases the apparent quantum yield of the reaction[12] and potentially lead to secondary reactions or photobleaching.[13] In particular for the synthesis of materials such as polymer conjugates,[1] functionalized surfaces,[14] microspheres,[15] sequence-defined macromolecules[16] or SCNPs,[17] competitive absorption presents a key challenge and limits the selectivity and control over the reaction. Herein, we present a strategy using electron deficient alkynes as dienophiles forming pro-fluorescent 1,4-dihydro-1-naphthols. The naphthols do not absorb in the wavelength range where the respective *o*-MBAs are activated. After the completion of the photoreaction, highly fluorescent naphthalenes are generated rapidly and quantitatively

by adding catalytic amounts of acid. The result is a photoreaction that is not disturbed by competitive absorption and is readily traceable by fluorescence spectroscopy due to the controlled formation of the fluorophore.
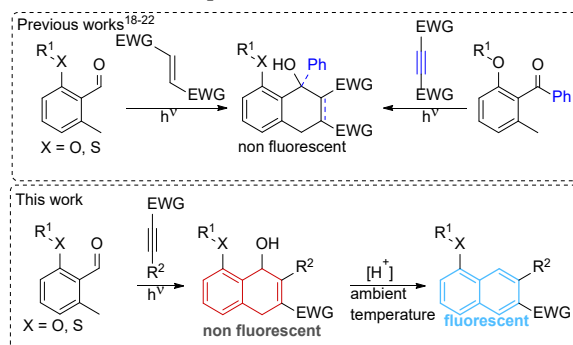
Scheme 1: (Top) Previous works using *o*-MBAs and electron deficient alkenes, or *o*-methylbenzophenones and alkynes.[18-22] (Bottom) Approach to fluorescent naphtalenes *via* non-fluorescent and blue-shifted 1,4-Dihydro-1-naphthols. EWG: electron withdrawing group.

The reaction of photocaged *o*-QDMs and electron deficient alkynes was first described by Porter and Tchir.[23] Wallace and coworkers investigated the reaction further and described the isolation of the naphthalenes after reflux in xylene with yields below 40%.[18] Based on this pioneering work, the reaction of 2-methyl benzophenones with alkynes was used frequently in synthesis, for instance to obtain 3-aroylchromones, benzo[*e*]pyrene bisimides and anthracene diimides using a vast excess of alkynes, strong acids, aprotic solvents and elevated temperatures.[19-21] Therefore, all the above mentioned reactions are of

limited use for the synthesis of advanced macromolecular architectures including acid or heat sensitive structures in particular for bioconjugates. Bioorthogonal reactions require an exceptional level of chemical selectivity and mild reaction conditions resulting in minimal interference with existing functional groups in biomolecules.[24] Furthermore, no heterobifunctional (non-symmetric) alkyne substrates have been reported for this reaction, rendering the synthesis of modular linker molecules unexplored. To overcome the limitations described above, we exploit the high driving force towards aromatization of 1,4-dihydro-1-naphtholes (DHNPs). We establish which substrates allow for efficient reaction under mild reaction conditions at concentrations suitable for polymer post-functionalization or bioligation (0.5 mmol L^{-1} to 50 mmol L^{-1}). Furthermore, we established synthesis strategies for heterobifunctional alkynes linkers. Initially, *o*-MBA (**A1**[25], 1.00 eq., 50 mmol L^{-1}) and the electron deficient acetylenedicarboxylic acid dimethylester (DMAD, **B2**, 1.05 eq, 52.5 mmol L^{-1}) are irradiated with a 385 nm LED in deuterated acetonitrile after deoxygenation. The formation of the intermediate DHNP (**C2**) was observed *via* ^{1}H-NMR (Figure 1). Upon exposure to air and therefore formation of carbonic acid by absorption of CO_2, the naphthalene **D2** was formed quantitatively within four hours *via* E1-elimination. Additionally, the conversion was directly followed by the disappearance of the UV-Vis *n*-π^* band at 320 nm of *o*-MBA and the appearance of a new absorption band at 280 nm

associated with the formation of **C2**. After on demand elimination, a new *n*-π^* band at 345 nm and a fluorescence band at 420 nm was observed, corresponding to **D2**. The absorption spectra impressively show that the intermediate **C2** does not interfere with the photoreaction as its absorption is shifted to shorter wavelengths. As the stability of **C2** was limited in ACN due to the extremely rapid elimination, ^{13}C and 2D-NMR spectra were recorded in C_6D_6 where **C2** was observed to be stable (see Supporting Figs. **S25-S26**). The substrate scope for the reaction was investigated using *o*-MBAs **A1** and **A2**, which exhibit distinctive absorption and reactivity and can be readily functionalized.[7] Alkynes **B1-B5** and diynes **B6-B7** are model substrates and functional analogues can be synthesized using different strategies. Terminal alkynes similar to methyl propiolate **B1** and phenylalkynes similar to **B3** can be readily prepared using propiolic acid or phenylpropiolic acid as precursors, respectively. The synthesis of functional analogues of dimethyl acetylenedicarboxylate (DMAD, **B2**) is considerably more difficult.[25] 4-oxo-2-butynoates **B4** and **B5** are readily accessible *via* Cu(I) mediated sp-sp^2 coupling of acid halides and propiolates.[26, 27] Symmetric and non-symmetric diynes **B6** and **B7** can be synthesized *via* different sp-sp coupling reactions and rearrangements.[28] Hay coupling appeared most attractive as proceeds in the absence of reactive organometallic compounds.[29] The synthesis and characterization of **A2, B4-B7** are described in the Supporting Information (Section 2.2-2.3). In Scheme 2, the observed reaction products are depicted and summarized in Table 1.
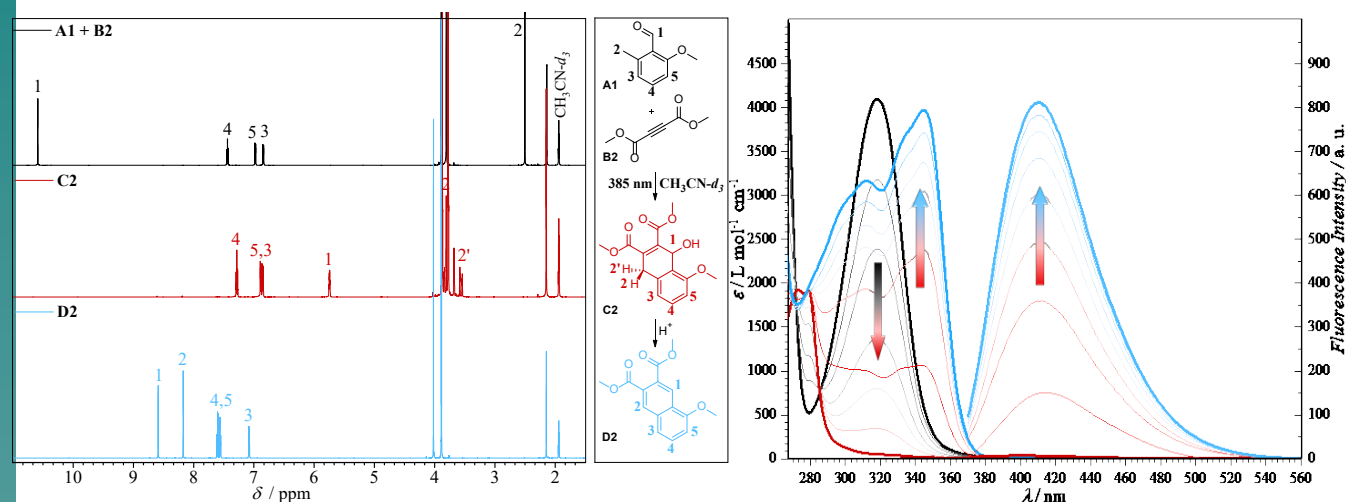
Figure 1 (Left) ^{1}H-NMR spectra of the reaction between *o*-MBA **A1** and **B2** to form cycloadduct **C2** under irradiation with 385 nm LED (LED-emission spectrum refer to supporting Fig. S69) and E1-Elimination to **D2** with assigned resonances. (Right) UV-VIS absorption and fluorescence emission spectra of the reaction mixture (black line), after up to 20 minutes of irradiation with 385 nm in the quartz cuvette (black to red line) and after addition of 10 mmol% *p*-TsOH and up to 12 minutes (red to blue line). For detailed analytical information for all compounds depicted in Fig. 1 refer to supporting Figs. S25, 26, 29, 30, 57, and 67.

If an alkyne with reduced reactivity is present, a fraction of *o*-MBA **A** is converted to benzocyclobutane **E** upon irradiation. This side-reaction was previously described when we studied the photoinduced [4+4]-cycloaddition of *o*-MBAs.[30]

Importantly, the ratio of Diels-Alder product **C/C'** and *4π* electrocyclization product **E** is concentration dependent (unimolecular *vs.* bimolecular reaction). Furthermore, regioisomers **C/C'** or **D/D'** can be formed in the case of non-symmetric alkynes.
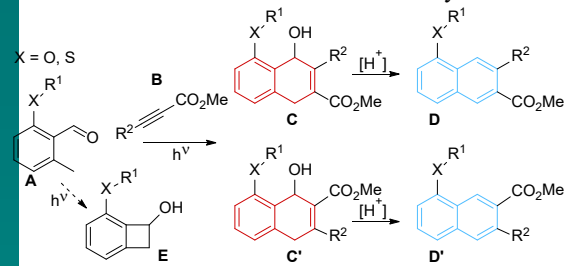
Scheme 2: Light induced reaction pathways of *o*-MBAs **A** in the presence of electron deficient alkynes **B** to form 1,4-dihydro-1-naphtholes **C** *via* Diels-Alder reaction or 1-hydroxybenzocyclobutanes **E** *via* [*4π*] electrocyclization. 1,4-dihydro-1-naphtholes **C** can be converted quantitatively to naphthalenes **D** with catalytic amounts of acid.

The experiments summarized in Table 1 were conducted in a batch reactor (setup described in supporting Fig. S1) to facilitate isolation and characterization *via* NMR spectroscopy and LCMS (supporting Figs. S27-55 and Figs. S56-S66). The two solvents, acetonitrile and toluene, were chosen to determine the influence of the dipole moment on the reaction. By comparing entry 1 and 2 or 3 and 4, it can be concluded that the ligation reactions of both *o*-MBAs with alkynes is more efficient in toluene. The more electron rich **B3** and to a lesser extent **B1** exhibit low reactivity and therefore result in the formation of benzocyclobutanes (see entries 1, 2, 5, 8). The same result can be observed comparing diynes **B6** and **B7** (entry 15, 16). *o*-MBA **A1** is more reactive than *o*-MBA **A2** resulting in a lower extent of benzocyclobutane formation (comparison of entries 3 and 7). In general, 4-oxo-2-butynoates **B4** and **B5** showed the highest reactivity and no *4π*-electrocyclization was observed as a result. The regioselectivity (ratio of **C/C'** and **D/D'**) is influenced by the steric hindrance and the electronic properties. For the substrate combination **A2** and **B4** as well as **A2** and **B5**, the resulting products **D8**, **D8'**, **D9** and **D9'** were separated and their structure elucidated *via* 2D-NMR spectroscopy. In addition, the reaction quantum yields for the reaction of **A1, A2** and **B4** were determined employing a monochromatic light source (refer to supporting Figs. S87-90). Whereas **A1** shows a minor dependence on the solvent dipole moment ($\Phi_{R,ACN}$: 0.68 ± 0.02 vs. $\Phi_{R,toluene}$: 0.78 ± 0.03), the reaction quantum yield of **A2** is highly solvent dependent ($\Phi_{R,ACN}$: 0.011 ± 0.001 vs. $\Phi_{R,toluene}$: 0.14 ± 0.01)
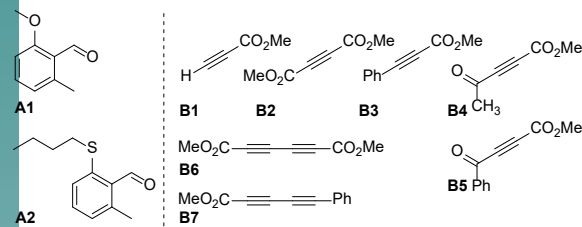
Scheme 3: Overview of the *o*-MBAs **A1, A2** and the alkynes **B1-7** utilized in the batch reactions.

Table 1: Batch photoligation experiments using *o*-MBAs **A1, A2** (5 mmol L^{-1}, 1.00 eq.) and alkynes **B1-B5** (5.5 mmol L^{-1}, 1.10 eq.) or diynes **B6-B7** (5.5 mmol L^{-1}, 1.10 eq.), irradiation time (t), Y^D isolated yield. **D/E**: ratio of the desired Diels-Alder adduct *vs.* the *4π* electrocyclization (refer to Scheme 2). **D/D'** ratio of regioisomers.

Nº	*o*-MBA	Alkyne	Solvent	t [min]	D/E	Y^D	D/D'
1	A1	B1	ACN	20	32:68	25%	100:0
2	A1	B1	Toluene	15	76:24	71%	100:0
3	A1	B2	ACN	20	n.d.	96%	--
4	A1	B2	Toluene	15	n.d.	97%	--
5	A2	B1	Toluene	30	5:95	--	100:0
7	A2	B2	ACN	30	13:87	--	--
6	A2	B2	Toluene	30	91:9	89%	--
8	A1	B3	Toluene	30	--	0%	--
9	A1	B4	ACN	30	n.d.	98%	70:30
10	A1	B4	Toluene	20	n.d.	97%	65:35
11	A1	B5	ACN	20	n.d.	95%	92:8
12	A1	B5	Toluene	15	n.d.	96%	87:13
13	A2	B4	ACN	40	97:3	94%^{a}	66:34
14	A2	B4	Toluene	20	99:1	96%^{a}	54:46
15	A2	B5	ACN	40	n.d.	94%^{a}	93:7
16	A2	B5	Toluene	20	n.d.	86%^{a}	76:24
17	A1	B6	Toluene	30	n.d.	96%	99:1
18	A1	B7	Toluene	30	55:45	49%	n.d.

a) combined yield after regioisomer separation

Most fluorophores depicted in Figure 2 do not have structural analogues reported in literature, therefore the fluorescence quantum yields were determined (refer to supporting Fig. S71). The emission wavelength of the naphthyl thioethers are in general higher than their naphthylether analogues (fluorescence spectra of **D1-D11** see supporting Figs. S72-S82). In addition, the photostability of the formed fluorophores was studied. **D1, D2, D7** and **D10** were irradiated under conditions similar to the photoligation (5 mmol L^{-1}, ACN-d^3, 385 nm LED and 365 nm LED respectively, NMR spectra see supporting Figs. S51-S55). **D1, D2** and **D7** showed high photostability, whereas **D10** was completely converted to various unknown products. This result underlines the advantage of the formation of a non-absorbing intermediate during the photoreaction, avoiding subsequent reaction or photobleaching.
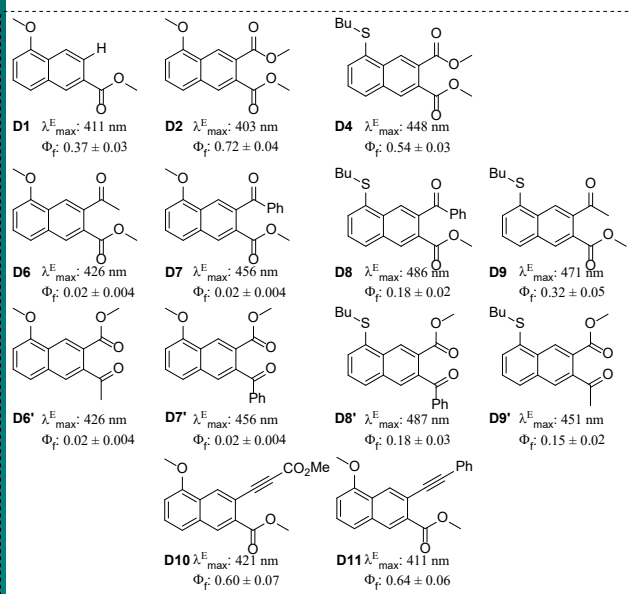
D1 λ^E_{max}: 411 nm
Φ_f: 0.37 ± 0.03

D2 λ^E_{max}: 403 nm
Φ_f: 0.72 ± 0.04

D4 λ^E_{max}: 448 nm
Φ_f: 0.54 ± 0.03

D6 λ^E_{max}: 426 nm
Φ_f: 0.02 ± 0.004

D7 λ^E_{max}: 456 nm
Φ_f: 0.02 ± 0.004

D8 λ^E_{max}: 486 nm
Φ_f: 0.18 ± 0.02

D9 λ^E_{max}: 471 nm
Φ_f: 0.32 ± 0.05

D6' λ^E_{max}: 426 nm
Φ_f: 0.02 ± 0.004

D7' λ^E_{max}: 456 nm
Φ_f: 0.02 ± 0.004

D8' λ^E_{max}: 487 nm
Φ_f: 0.18 ± 0.03

D9' λ^E_{max}: 451 nm
Φ_f: 0.15 ± 0.02

D10 λ^E_{max}: 421 nm
Φ_f: 0.60 ± 0.07

D11 λ^E_{max}: 411 nm
Φ_f: 0.64 ± 0.06

Figure 2: Isolated products from the photoligation experiments summarized in Table 1, including fluorescence emission maxima and fluorescence quantum yields.

As reported earlier, *o*-MBA thioaldehydes such as **A2** are performing reasonably well in protic solvents including solvent mixtures containing water.[7] The compatibility with aqueous solutions is crucial for applications involving biomolecules. Surprisingly, we found that the reaction of **A2** in ACN:H2O mixtures results in the decrease of *4π* electrocyclization compared to pure acetonitrile (refer to Table 2). Although, longer reaction times due to the previously reported decrease in the quantum yield of the photoreaction were required.[31] In addition, from the ACN:H2O reaction mixture we performed the elimination reaction in a buffer solution at pH 4.2 and monitored the reaction progress *via* fluorescence spectroscopy (refer to supporting Fig. S8). The fact that the elimination takes place at ambient temperature in aqueous solution without the addition of a catalyst is especially important considering potential biological applications.

Table 2: Batch photoligation experiments in ACN:H2O (75:25 *v/v*) using *o*-MBA **A2** (5 mmol L^{-1}, 1.00 eq.) and alkyne **B2** or **B4** (5.5 mmol L^{-1}, 1.10 eq.) LED-irradiation wavelengths (λ_{max}), irradiation time (t), Y^D isolated yield. **D/E**: ratio of the desired Diels-Alder adduct *vs.* the *4π* electrocyclization (see Scheme 2). **D/D'** ratio of regioisomers.

N°	*o*-MBA	Alkyne	λ_{max}[nm]	t [min]	$R^{D/E}$	Y^D	D/D'
1	A2	B2	385	150	98:2	95%	--
2	A2	B2	415	360	86:14	75%	--
3	A2	B4	415	360	100:0	98%	82:18

In order to use the herein established reactions, it is essential to have access to functional alkyne-linkers. Importantly, alkynoates are reactive towards many nucleophiles. Therefore, we designed two linkers (**L1**, **L2**) that can be attached to substrates using well-established ligation methods, such as esterification. **L1** is prepared *via* esterification of propiolic acid, whereas **L2** is accessible by ring opening of *ε*-caprolactone with methyl propiolate following a literature protocol.[32]
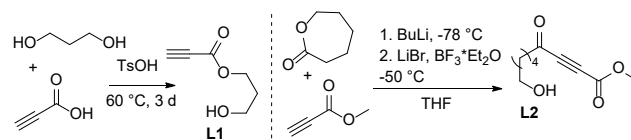
Scheme 4: Synthesized linkers **L1**, **L2** exhibiting terminal OH groups for further functionalization (characterization supporting Figs. S17-S20).

In conclusion, we pioneer a light induced ligation system for *o*-MBAs that allows for gated fluorescence response. The self-reporting can be triggered quantitatively under very mild conditions, allowing for fluorometric evaluation of the ligation reaction. With the most suitable alkynoate substrates, we designed a linker molecule that can be attached to payload molecules and demonstrated that the reaction can be carried out in aqueous media using visible light. We envisage that this technique will open new possibilities in biological applications and advanced material synthesis.

ASSOCIATED CONTENT

Supporting Information

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Notes
The authors declare no competing financial interests.

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