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Discovery of Long-Wavelength Photoactivatable Diaryltetrazoles for Bioorthogonal 1,3-Dipolar Cycloaddition Reactions

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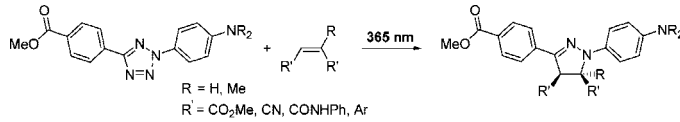
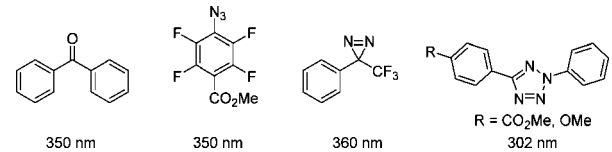
ABSTRACT



Several long-wavelength (365 nm) photoactivatable diaryltetrazoles were discovered by screening a series of substituted diaryltetrazoles and subsequently showed excellent reactivity in the photoactivated 1,3-dipolar cycloaddition reactions toward electron-deficient and conjugated alkenes in organic solvents as well as an alkene-containing protein in the aqueous buffer.

Photoactivatable reagents have been widely used as a photocrosslinking tool in mapping specific ligand binding regions within large proteins, characterizing receptor dynamics in the lipid membrane, and capturing transient protein−protein and protein−DNA interactions.[1] Because photoactivatable reagents are inert under normal conditions and become activated upon UV irradiation, they offer a unique advantage of allowing temporal and spatial control over reactivity. The most commonly used photoactivatable groups include aryl ketones,[2] aryl azides,[3] and diazirines,[4] which generally require photoirradiation at wavelength <365 nm in generating their respective biradical, nitrene, and carbene species (Scheme 1). These reactive intermediates then undergo rapid C−H (or X−H) insertion reactions with the surrounding biomolecules to form the cross-linked photoadducts.[1b]

Scheme 1. Representative Photoactivatable Groups and Their Respective Photoactivation Wavelength



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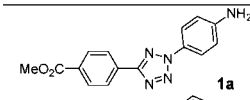
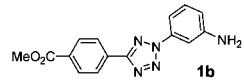
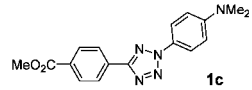
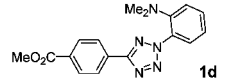
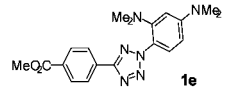
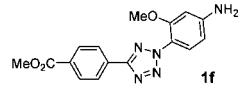
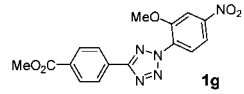
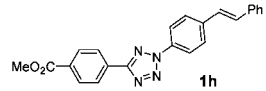
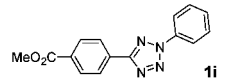
We recently reported a new class of photoactivatable groups responsive to 302 nm handheld UV light based on the diaryltetrazole moiety (Figure 1).[5] Unlike other photoactivatable groups which generally exhibit poor chemoselectivity, diaryltetrazoles showed selective reactivity toward alkenes in the photoinduced 1,3-dipolar cycloaddition reaction both in vitro and in living cells.[6] Because of their potential as chemical reporters in living systems,[7] it is highly desirable that their photoactivation takes place at longer wavelength such that it causes minimum photodamage to the living cells and organisms.[8] Herein, we report the discovery of several 365-nm UV photoactivatable diaryltetrazoles and the subsequent characterization of their reactivity in the photoactivated 1,3-dipolar cycloaddition reactions toward a series of small-molecule alkenes in organic solvents as well as an alkene-containing protein in the biological buffer.

The rate of photolysis at any given wavelength in a photochemical reaction is proportional to quantum yield and molar absorption coefficient at the irradiation wavelength.[9] Since the quantum yields for the photolysis of diaryltetrazoles are very high in the range of 0.5−0.9,[10] we reasoned that long-wavelength photoactivatability can be achieved by placing auxochromic and conjugative substituents on the *N*-phenyl ring to increase molar absorption at the long-wavelength region. Thus, a series of diaryltetrazoles (**1a**−**h**) were synthesized,[11] and their UV−vis spectroscopic properties were collected in Table 1. Compared to simple diaryltetrazole **1i**, the attachment of either an auxochrome such as $-NH_2$ (**1a**) and $-NMe_2$ (**1c**) or conjugative groups such as styryl (**1h**) at the para position of the *N*-phenyl ring led to significant bathochromic shifts in λ_{max} (34 nm for **1a**, 60 nm for **1c**, 28 nm for **1h**), with concomitant increases in the molar absorption coefficients at 365 nm. On the other hand, attachment of auxochromes at the ortho position caused hypsochromic shifts in λ_{max} (**1d**−**g**). However, the molar absorption coefficients increased substantially for **1e** and **1g** compared to **1i** due to peak broadening.

To directly assess the long wavelength photoreactivity of these diaryltetrazoles toward alkene dipolarophiles, we incubated them with methyl methacrylate in ethyl acetate and irradiated the mixtures with a handheld UV lamp at either 302 nm (UVP, model UVM-57, 0.16 AMPS) or 365 nm (UVP, model UVGL-25, 0.16 AMPS) for 2 h. Under both conditions, only one regioisomer of the pyrazoline cycloadducts (**3**) was observed in crude products based on the NMR signals of the pyrazoline ring protons. Generally, 302-nm irradiation afforded higher yields than 365-nm irradiation for nearly all diaryltetrazoles tested (Table 2). This can be attributed to the filtering effect[12] as the pyrazoline products absorb light strongly at longer wavelength region ($\lambda_{max} \approx$ 370 nm). Under 365-nm photoirradiation, diaryltetrazoles bearing amino and dimethylamino at the para position of the *N*-phenyl ring gave rise to excellent conversions (entries 1 and 3 in Table 2), consistent with their spectroscopic properties (Table 1). By contrast, *m*-amino- (**1b**) and *o*-dimethylamino-substituted (**1d**) diaryltetrazoles gave significantly lower conversions (compare entry 2 to 1 and entry 4 to 3). Attachment of additional auxochromes at the ortho position of the *N*-phenyl ring led to a substantial drop in long wavelength photoreactivity but not in short wavelength photoreactivity (compare entry 5 to 3 and entry 6 to 1). In addition, placement of a strong electron-withdrawing group on the *N*-phenyl ring significantly reduced the long wavelength photoreactivity (compare entry 7 to 6). Furthermore, placement of a styryl group at the para position of the *N*-phenyl ring afforded 50% conversion upon 365-nm photoactivation (entry 8). Taken together, it appears the photoreactivity of diaryltetrazoles is largely dependent on

Table 1. Absorption Maxima and Molar Absorption Coefficients for Various Diaryltetrazoles

diaryltetrazole	λ_{max} (nm)	ε_{302} ($M^{-1}cm^{-1}$)	ε_{365} ($M^{-1}cm^{-1}$)
 **1a**	310	20,500	3,500
 **1b**	268	14,000	600
 **1c**	336	20,000	18,700
 **1d**	258	2,900	400
 **1e**	258	15,300	7,600
 **1f**	254	15,900	1,000
 **1g**	264	13,400	3,400
 **1h**	304	4,700	2,100
 **1i**	276	7,100	900

[a] UV−vis was measured by dissolving tetrazoles in MeOH/H_2O (1:1) mixed solvent to derive the final concentrations of 25 μM.

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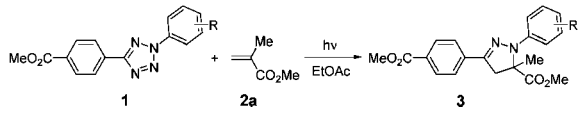
Table 2. Photoactivated 1,3-Dipolar Cycloaddition of **1** and **2a** at Two Different Wavelengths



entry	diaryltetrazole	yield (%)[a] at 302 nm	conversion (%)[b] at 365 nm
1	R = *p*-NH_2 (**1a**)	84	90
2	R = *m*-NH_2 (**1b**)	26	<5
3	R = *p*-NMe_2 (**1c**)	93	95
4	R = *o*-NMe_2 (**1d**)	72	40
5	R = *o*, *p*-$(NMe_2)_2$ (**1e**)	95	70
6	R = *o*-OMe-*p*-NH_2 (**1f**)	91	45
7	R = *o*-OMe-*p*-NO_2 (**1g**)	92	15
8	R = *p*-styryl (**1h**)	94	50

[a] Reactions were conducted by irradiating 20 mg of **1** and 5 equiv of **2a** in 6 mL of EtOAc with a 302-nm UV lamp in quartz test tubes for 4 h. The yields were isolated yields. [b] Reactions were conducted by irradiating 1 mg of **1** and 5 equiv of **2a** in 2 mL of EtOAc with a 365-nm UV lamp in quartz test tubes for 2 h. Conversions were estimated based on crude NMR spectra after removal of excess **2a**.

their UV−vis properties. It is noteworthy that 365-nm photoirradiation of the parent diaryltetrazole **1i** did not yield any observable pyrazoline cycloadduct.

To examine the reactivity of the nitrile imine dipoles derived from the long-wavelength photoactivatable diaryltetrazoles, we performed the photoactivated 1,3-dipolar cycloaddition reactions by incubating **1a** and **1c** with a range of alkene dipolarophiles (Table 3). As expected, electron-deficient alkenes, such as methyl methacrylate, *N*-phenyl-methacrylamide, and acrylonitrile, reacted efficiently with **1a** upon 365-nm photoirradiation, affording the 5-substituted pyrazoline adducts (entries 1−3). Unexpectedly, reaction with methyl acrylate gave rise to the oxidized pyrazole product with 90% isolated yield (entry 4). Furthermore, styrene derivatives generally serve as good dipolarophiles for **1a**, with the reactivity trend paralleling the electron-withdrawing ability of the para substituents (entries 5−8). For the dimethylamino-substituted diaryltetrazole (**1c**), electron-deficient alkenes such as methyl methacrylate, acrylonitrile, and *N*-methyl-maleimide reacted well (entries 9−11) while styrene gave a modest yield (entry 12).

To understand how the attachment of auxochromes leads to long-wavelength photoactivatability, we solved the X-ray structure of 2-(4′-methoxyphenyl)-5-(2″-isopropoxy-4″-methoxyphenyl)tetrazole (**4**, Figure 1). A quick glance of the structure revealed that three aromatic rings are perfectly coplanar with the dihedral angles for N(1)−C(1)−C(2)−C(3) and C(17)−C(12)−N(2)−N(1) to be 8° and −6°, respectively. This is not surprising given that the tetrazole ring does not bear a single hydrogen and thus has no steric repulsions with hydrogens from the neighboring phenyl rings. This arrangement allows the lone pairs of auxochromes (e.g., **1a**

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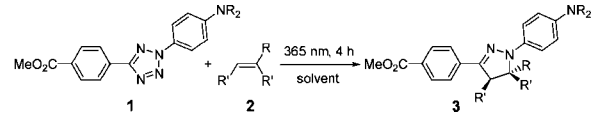
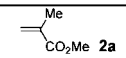
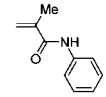
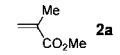
Table 3. Photoactivated 1,3-Dipolar Cycloaddition of **1** with Various Alkene Dipolarophiles at 365 nm



entry	diaryltetrazole	alkene	yield (%)[b]
1	**1a**	 **2a**	84
2	**1a**	 **2b**	95
3	**1a**	 **2c**	81
4	**1a**	 **2d**	90[c]
5	**1a**	 **2e**	93
6	**1a**	 **2f**	95
7	**1a**	 **2g**	81
8	**1a**	 **2h**	74
9	**1c**	 **2a**	93
10	**1c**	 **2c**	100
11	**1c**	 **2i**	90
12	**1c**	 **2e**	53

[a] Reactions were conducted by irradiating 20 mg of **1a** or **1c** and 5 equiv of **2** in 6 mL of benzene/EtOAc (1:2) in quartz test tubes for 4 h. [b] Isolated yields. [c] Oxidized pyrazole product was isolated.

and **1c**) at the para position to maximally interact with the tetrazole π system, resulting in bathochromic shifts. It is plausible that attachment of substituents at the ortho position alters this planar arrangement, resulting in hypsochromic shift. However, it remains unclear whether substituents on the *N*-phenyl ring also directly weaken the N^2-N^3 σ bond, the first step during the rupture of the tetrazole ring.[13]

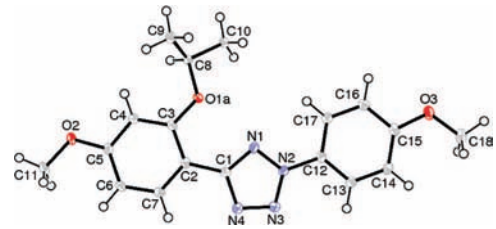


Figure 1. ORTEP representation of tetrazole **4** structure.

To examine whether long-wavelength photoactivatable diaryltetrazoles can be used to label an alkene-containing protein in biological buffer, we incubated six long-wavelength photoactivatable diaryltetrazoles (**1a**,**c**−**f**,**h**) with

lysozyme acylated with methacrylic anhydride. The mixtures were photoirradiated with a handheld 365 nm UV lamp for 20 min. Because the pyrazoline cycloadducts are fluorescent,[6a] we performed an in-gel fluorescence analysis to check for the cycloadduct formation (Figure 2). Pairwise

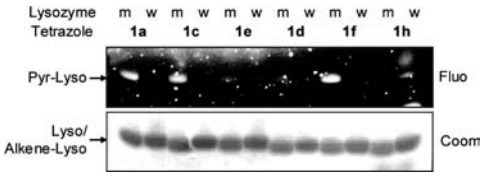


Figure 2. Photoactivated 1,3-dipolar cycloaddition of diaryltetrazoles with either alkene-modified lysozyme (m) or wild-type lysozyme (w) at 365 nm: top panel, in-gel fluorescence; bottom panel, Coomassie blue staining.

comparisons of the reactions involving either the alkene-modified lysozyme or the wild-type lysozyme revealed that three diaryltetrazole (**1a**, **1c**, **1f**) showed specific cycloadduct formation. To our satisfaction, diaryltetrazoles that exhibited the highest photoreactivity in the earlier study (**1a** and **1c**; Table 2) showed the strongest fluorescent intensity in this analysis. Interestingly, a modest photoactivatable diaryltetrazole (**1f**) also gave rise to strong fluorescence on the SDS-PAGE, which can be partly explained by its relatively higher quantum efficiency during the ring opening step.[14]

In summary, we have identified several long-wavelength photoactivatable diaryltetrazoles that showed excellent reactivity toward electron-deficient and conjugated alkenes in organic solvents and an alkene-containing protein in the aqueous buffer. Compared to the 302-nm photoactivatable diaryltetrazoles we reported previously,[5] these new diaryltetrazole derivatives should serve as safer and more convenient photoactivatable reagents for applications of bioorthogonal 1,3-dipolar cycloaddition reactions in living systems.

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Supporting Information Available: Experimental details and characterization of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) The quantum yields for **1a**, **1c**, and **1f** were determined to be 0.006, 0.04, and 0.03, respectively; see the Supporting Information for details.