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Discovery of new photoactivatable diaryltetrazoles for photoclick chemistry via 'scaffold hopping'

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ABSTRACT

We report the discovery of two long-wavelength (365 nm) photoactivatable diaryltetrazoles through screening a small library of diaryltetrazoles that were designed using a 'scaffold hopping' strategy. A naphthalene-derived tetrazole showed excellent reactivity in the photoinduced cycloaddition reaction with methyl methacrylate under 365 nm photoirradiation in acetonitrile PBS buffer mixture. Besides, the brightly fluorescent pyrazoline cycloadducts that were formed further increase the potential utility of these new diaryltetrazoles as 'photoclick' reagents and as reporters in biological studies.

Light-induced chemical reactions regulate many important cellular and organismal functions in nature. For example, prokaryotes such as *Brucella abortus* employ a photoinduced flavin addition to cysteine of a histidine kinase to regulate bacterial chemotaxis, phototaxis, and virulence.[1] In mammals, a photoinduced isomerization of 11-*cis*-retinyl Schiff base in rhodopsin forms the chemical basis for vision formation.[2] To harness the power of light for cell biology studies, natural sensory photoreceptors such as channel rhodopsins have been successfully used as optogenetic modules in order to achieve photo-regulation of the function of neurons and other cells.[3] However, because fusion of large photo-sensing proteins adds considerable mass to a target protein, an alternative approach has involved the introduction of small photoreactive chemical moieties, for example, a photocaged[4] or a photoisomerizable group,[5] to a target protein site-specifically followed by subsequent light-dependent functional manipulation.

Recently, we have developed a protein photo-modification protocol based on a bioorthogonal, photoinduced tetrazole-alkene cycloaddition reaction ('photoclick chemistry').[6] In this approach, a bioorthogonal alkene[7] or tetrazole[8] is introduced site-selectively into proteins, which can be subsequently modified with the cognate reagents in live cells in a light-dependent manner. Since alkene structures are considerably smaller in size compared to tetrazoles, it is more straightforward to encode alkenes in proteins and use tetrazoles as the reagents to drive the protein photo-modification. To this end, robust tetrazole reagents need to be identified that show both high reactivity[9] and long-wavelength photoactivatability.[10] In addition, it is also desirable that the pyrazoline cycloadducts exhibit bright fluorescence in order to facilitate the monitoring of protein photo-modification in vivo. Here we report the synthesis of a small library of tetrazoles and subsequent identification of two new diaryltetrazoles that show 365 nm photoactivatability and cycloaddition products with good fluorescent properties.

Our previous study has identified several 365 nm photo-reactive tetrazoles containing either amino or styryl group at the *para*-position of the *N*-phenyl ring.[10] The long-wavelength photoreactivity was attributed to greater absorption at the long-wavelength region in the UV–vis spectra. However, the pyrazoline cycloadducts derived from the amino-substituted diphenyltetrazole showed rapid photo-bleaching, limiting its utility in biological studies. To overcome this problem, we borrowed a concept from medicinal chemistry dubbed as 'scaffold hopping', where key pharmacophores are allowed to 'jump' from one scaffold to another in order to derive novel ligands with improved bioactivities.[11] We hypothesized that by 'hopping' the photoreactive tetrazole moiety from aniline to other known long-wavelength UV absorbing scaffolds, we may obtain new tetrazoles with increased photoreactivity and photostability.

Based on the long-wavelength UV absorbance and synthetic accessibility, five chromophores were selected as alternative scaffolds for our new diaryltetrazoles **1**–**5** (Fig. 1). Starting from 2-naphthalenediazonium salt, the 2-naphthoate tetrazoles **7** and **11** were efficiently prepared using the Kakehi procedure,[12] with the yield of 26% and 62%, respectively (Scheme 1). Following

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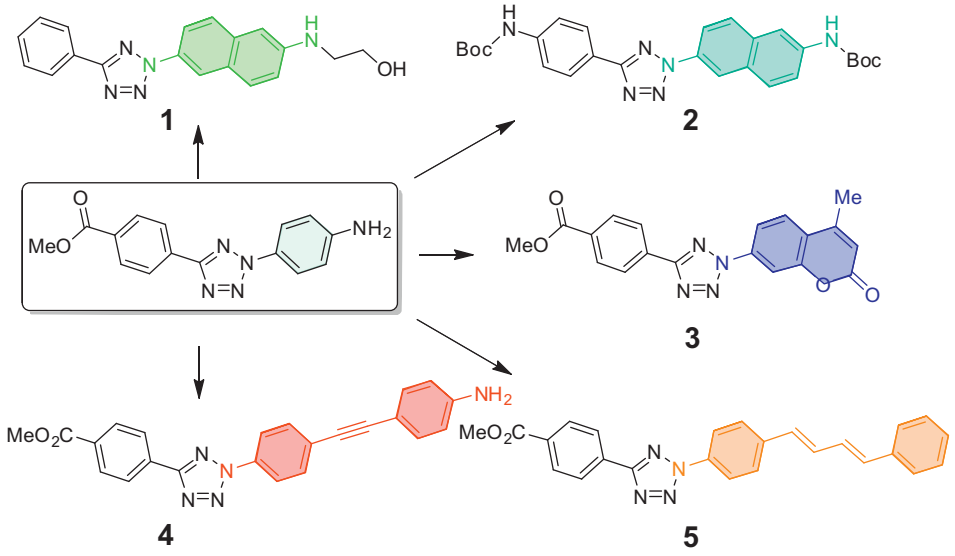
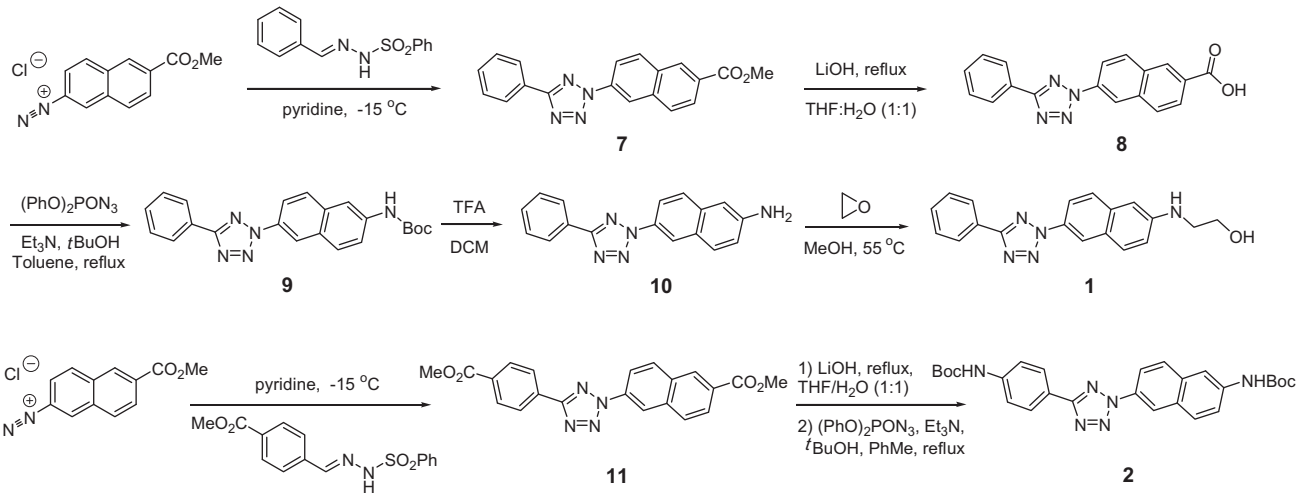


Figure 1. Structures of diaryltetrazoles with alternative scaffolds.



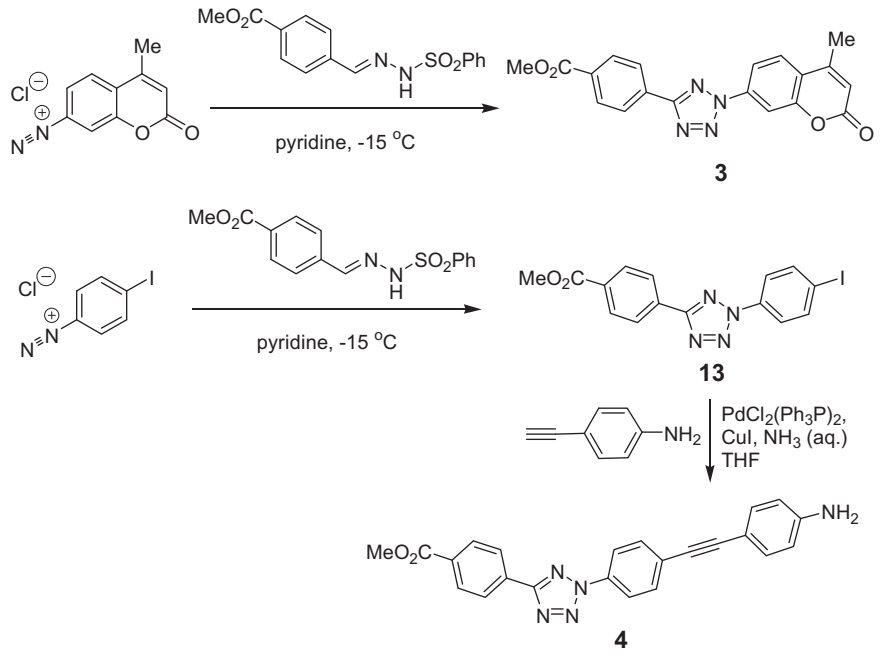
Scheme 1.

LiOH-mediated hydrolysis, the tetrazole carboxylic acids were converted to the Boc-protected-6-aminonaphthyl-tetrazoles **9** and **2** via Curtius rearrangement in 67% and 46% yield, respectively. Subsequent deprotection of **9** with TFA followed by alkylation of naphthylamine with ethylene oxide produced a water-soluble tetrazole **1**. However, deprotection of **2** generated a chemically unstable compound so we decided to use the Boc-protected **2** directly in our reactivity studies.

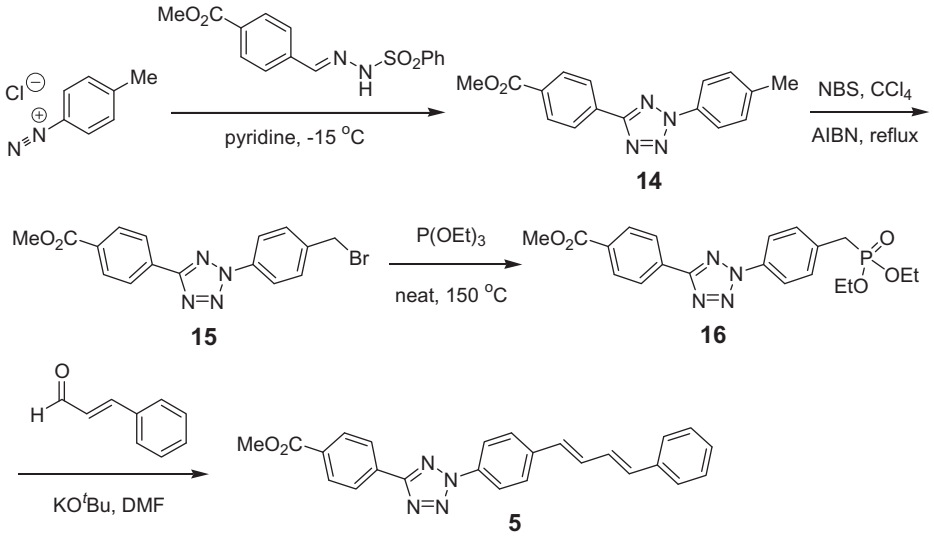
Since coumarin is a blue fluorophore and absorbs strongly in the UV region around 365 nm, we prepared a tetrazole-modified coumarin **3** from the commercially available 7-amino-4-methylcoumarin with a yield of 81% (Scheme 2). Separately, since diarylacetylene and diaryl-1,3-butadiene have been used in fluorophore design,[13] we appended the photoreactive tetrazole to these scaffolds and generated tetrazoles **4** and **5**. Tetrazole **4** was readily prepared from the Sonogashira coupling between 2-*p*-iodophenyltetrazole **13** and *p*-ethynylaniline (Scheme 2). On the other hand, the 1,4-dipenylbutadiene-conjugated tetrazole **5** was synthesized in four steps: (i) Kakehi tetrazole synthesis produced *N*-tolyltetrazole **14** in 86% yield; (ii) bromination of **14** with NBS and catalytic amount of AIBN gave the intermediate **15** in 80% yield; (iii) heating of benzyl bromide **15** in triethylphosphite gave benzyl phosphonate **16** in 60% yield; and (iv) the Horner–Wadsworth–Emmons reaction between **16** and cinnamaldehyde afforded the final product tetrazole **5** in 39% yield (Scheme 3).

Since tetrazole photoreactivity at any given wavelength is dependent on molar absorption coefficient at that particular wavelength,[14] the UV–vis spectra of tetrazoles **1**–**5** were taken (Fig. S1 in Supplementary data) and the data were collected in Table 1. Compared to *p*-aminodiphenyltetrazole, all five tetrazoles showed significant bathochromic shift in λ_{max}, ranging from 14 nm for tetrazole **2** to 50 nm for tetrazole **5**. The strong absorption bands in 300–400 nm can be attributed to the π–π^* electronic transition between HOMO and LUMO orbitals of the tetrazole-conjugated fluorophores. The extent of bathochromic shift appears to correlate with the calculated HOMO–LUMO gap as tetrazoles **4** and **5** with smaller gap showed larger λ_{max} shifts.

To probe whether long-wavelength UV absorption leads to enhanced photoreactivity in biological systems, we initially attempted to test the reactivity of all five tetrazoles in a mixed acetonitrile/PBS buffer (1:1), pH 7.5, and found that tetrazoles **1** and **2** and their cycloaddition photoproducts were reasonably soluble but tetrazoles **3**–**5** were insoluble. Therefore, we performed a fluorescence-based screen of the photoreactivity of tetrazoles **1**



Scheme 2.



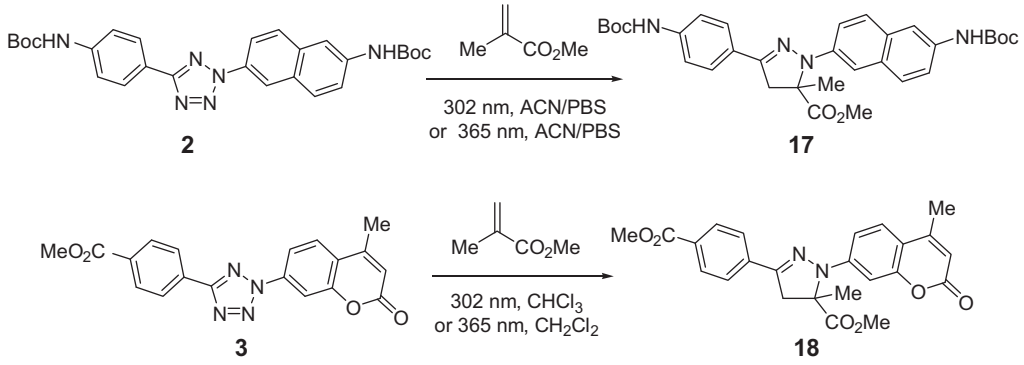
Scheme 3.

Table 1
Absorption maxima, molar absorption coefficients and molecular orbital energy calculation for diaryltetrazoles[a]

Tetrazole	λ_{max}[b] (nm)	ε_{302} (M^{-1} cm^{-1})	ε_{365} (M^{-1} cm^{-1})	ε_{395} (M^{-1} cm^{-1})	HOMO[d] (eV)	LUMO[d] (eV)	$\Delta_{LUMO-HOMO}$[d] (eV)
p-amino[c]	310	20500	3500	N.D.	−5.66	−1.51	4.15
1	338	7103	11908	1935	−5.64	−1.40	4.24
2	324	16415	1813	1042	−5.57	−1.40	4.17
3	332	25838	907	682	−6.57	−2.22	4.35
4	346	28892	35177	6167	−5.41	−1.74	3.67
5	360	9035	30819	8184	−5.60	−1.99	3.61

[a] UV–vis was measured by dissolving tetrazoles in $CHCl_3$ to derive the final concentration of 25 μM.
[b] λ_{max} were derived from the wavelength region of 300–450 nm.
[c] Data were taken from Ref. 10
[d] DFT calculation were performed at the B3LYP/6-31G* level in vacuum using the SPARTAN'08 program.

and **2** under 302, 365, or 395 nm UV irradiation in the aqueous buffer by taking advantage of the fact that pyrazoline cycloadducts are fluorescent. We found that tetrazole **2** showed rapid 'turn-on' fluorescence upon 302 and 365 nm photoirradiation (Fig. 2) while tetrazole **1** showed no change in fluorescence after the photoirradiation even though tetrazole **1** itself was weakly fluores-



Scheme 4.

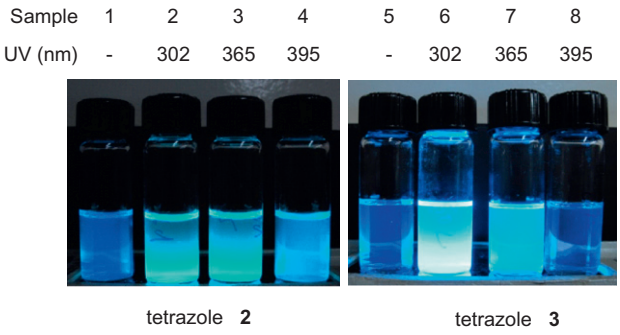


Figure 2. Fluorescence images of tetrazoles **2** and **3** after the photo-irradiation with methyl methacrylate in acetonitrile/PBS buffer (1:1) and chloroform, respectively; λ_{ex} = 365 nm. The concentrations of tetrazole and methyl methacrylate were 100 μM and 10 mM, respectively, and the duration of photoirradiation was 10 min.

cent (Fig. S2). To gain insight about the intrinsic photoreactivity of tetrazoles **3**–**5**, we dissolved these compounds in chloroform and examined their reactivity towards methyl methacrylate under 302, 365 or 395 nm irradiation. Interestingly, the coumarin-fused tetrazole **3** showed bright 'turn-on' fluorescence after exposure to the 302 or 365 nm lamp (Fig. 2); tetrazoles **4** and **5** were brightly fluorescent before and after the photoirradiation and the TLC monitoring showed no detectable new spots (Fig. S3).

To confirm the fluorescence screening results, we carried out the photoinduced cycloaddition reactions with tetrazoles **2** and **3** on a preparative scale (Scheme 4). For tetrazole **2** under 302 nm UV irradiation for 2 h, the expected pyrazoline product **17** was obtained in 89% isolated yield. Using the purified **17** as a positive control in the HPLC-based analysis (Fig. S4), the same reaction on an analytical scale showed a conversion of 90% under 365 nm photoirradiation for only 5 min. Similarly, the pyrazoline product **18** was isolated in 68% yield after tetrazole **3** was irradiated at 302 nm in chloroform for 1.5 h, and 13% yield when 365 nm UV lamp was used for 6 h. The reduced yield seen at 365 nm was presumably due to the significantly lower molar absorption coefficient at 365 nm compared to 302 nm (Table 1).

In summary, we have identified two long-wavelength photoactivatable diaryltetrazoles containing either naphthalene or coumarin chromophore. The naphthalene-derived tetrazole showed excellent long-wavelength photoreactivity in an acetonitrile/PBS mixed solvent. Additionally, both tetrazoles produced bright pyrazoline fluorophores upon the cycloaddition reactions, which may prove critical for their use as 'photoclick' reagents in visualizing and perturbing the alkene-tagged proteins in living cells.[7] Detailed characterization of these two compounds and additional structural modifications are currently underway and will be reported in due course.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.04.087.

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