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Synthesis and Evaluation of Photoreactive Tetrazole Amino Acids

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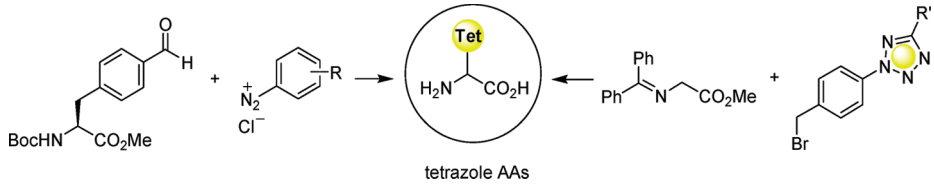
ABSTRACT



tetrazole AAs

Six photoreactive tetrazole amino acids were efficiently synthesized either by the de novo Kakehi tetrazole synthesis method or by alkylation of a glycine Schiff base with tetrazole-containing alkyl halides, and four of them showed excellent reactivity toward a simple alkene in the photoinduced 1,3-dipolar cycloaddition reaction in acetonitrile/PBS buffer (1:1) mixture.

Photoreactive reagents have been widely used in biochemistry and molecular biology in mapping the interfaces of ligand−receptor and protein−protein interactions.[1] Since photoreactive reagents are "silent" under normal conditions and become chemically reactive only upon UV irradiation, they offer a spatial and temporal control of the ligand or protein function.[2] One major approach in exerting photochemical control in protein systems is to incorporate photoreactive amino acids into proteins site-selectively using either the Amber codon suppression strategy or the metabolic incorporation strategy.[3] To this end, a number of photoreactive amino acids have been successfully developed, e.g., *p*-benzoyl-L-Phe (*p*BPA),[4] *p*-azido-L-Phe (*p*Azpa),[5] *p*-3-(trifluoromethyl)-3*H*-diazirin-3-yl-L-Phe (*p*TmdPhe),[6] *o*-nitrophenylalanine (*o*NPA),[7] photoleucine, and photomethionine[8] (Figure 1). Although these reagents have provided invaluable photochemical tools for protein functional studies, additional chemical moieties with novel photoreactivity are still needed in order to further expand the capability of photoregulation.

We have recently reported a new class of photoreactive reagents, 2,5-diaryltetrazoles,[9] with exquisite reactivity toward alkenes both *in vitro* and in living cells.[10] Upon photoirradiation, unlike previously known photoreactive

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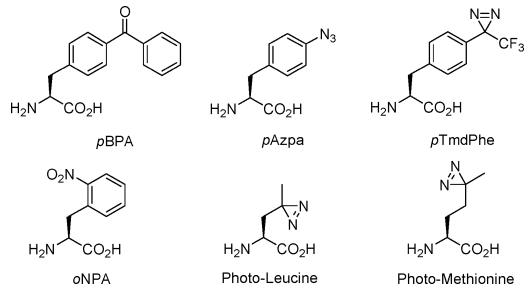
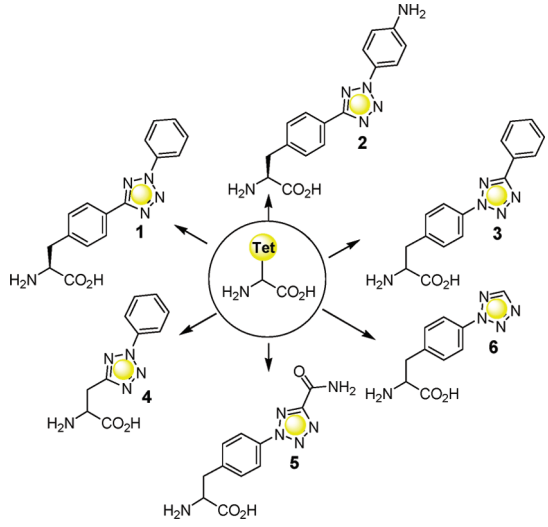


Figure 1. Representative photoreactive amino acids.

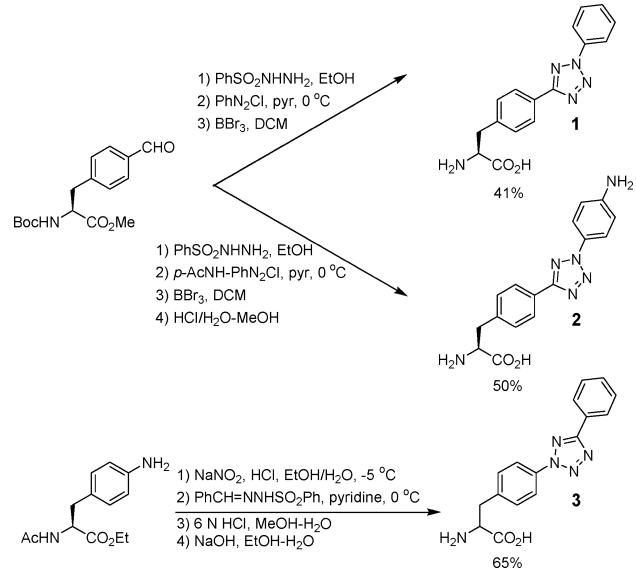
motifs (Figure 1) that produce highly reactive intermediates (radical, nitrene, or carbene)[11] that undergo nonselective C−H (or X−H) insertion, tetrazoles generate significantly more stable nitrile imine dipoles that selectively react with suitable alkenes in solution via 1,3-dipolar cycloaddition. In essence, the tetrazole moiety provides a new photoregulation modality, i.e., photo modification, by which a multitude of alkene-tethered biophysical and biochemical probes can be attached to the tetrazole site through a photoinduced 1,3-dipolar cycloaddition reaction. To enable rapid access of this novel photoregulation modality, herein we report the first synthesis of six tetrazole amino acids (Scheme 1) and the evaluation of their reactivities toward an alkene dipolarophile under a mild photoinduced 1,3-dipolar cycloaddition condition in acetonitrile/PBS buffer (1:1) mixture.

Scheme 1. Structures of Tetrazole Amino Acids **1**−**6**



We initially focused on the synthesis of 2,5-diaryl tetrazole-based amino acids **1**−**3** (Scheme 1) because (i) diaryltetrazoles exhibited excellent photoreactivity in aqueous buffers in our previous studies,[9] and (ii) Kakehi tetrazole synthesis offers a facile route for the preparation of diaryltetrazoles from aryl aldehydes and arene diazonium salts.[12] Two types of diaryltetrazole amino acids were designed: type I, with the amino acid motif attached to the C5-aryl ring (**1**, **2**), and type II, with the motif attached to the N2-aryl ring (**3**) (Scheme 1). In a convergent manner, type I tetrazole amino acids can be synthesized from arene diazonium salts and *p*-formyl-L-phenylalanine, which was readily prepared via the palladium-catalyzed hydroformylation of a protected tyrosine triflate.[13] When simple phenyldiazonium chloride was used, an optically pure diphenyltetrazole amino acid **1** was obtained with an overall 41% yield in three steps (Scheme 2). Since our previous studies indicated that the amino substitution at the *para* position of the N-aryl ring of 2,5-diaryltetrazoles generates long-wavelength photoactivatable tetrazoles,[9b] we also prepared *p*-amino-diphenyltetrazole amino acid **2** in four steps with an overall yield of 50% from *p*-formyl-L-phenylalanine and acetamidophenyl diazonium chloride (Scheme 2).

Scheme 2. Synthesis of Diaryltetrazole Amino Acids **1**−**3**



On the other hand, type II tetrazole amino acid **3** was synthesized in a similar manner except that the amino acid moiety was attached to phenyl diazonium chloride, which was formed smoothly from a protected 4-amino-phenylalanine derivative. Kakehi tetrazole synthesis with benzaldehyde followed by deprotection gave the tetrazole amino acid **3** with an excellent yield of 65% over four steps (Scheme 2).

Although a number of unnatural amino acids carrying

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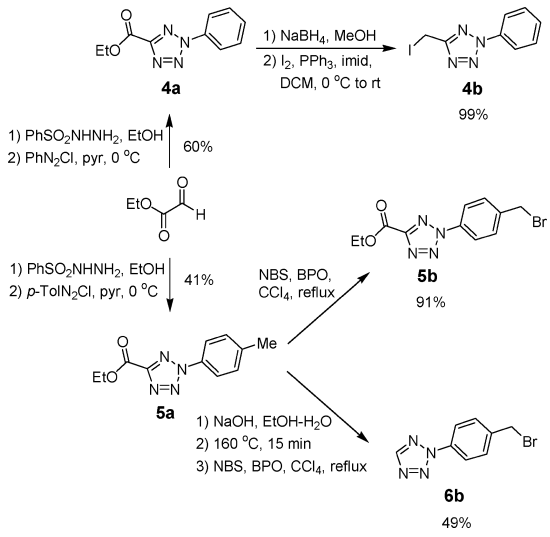
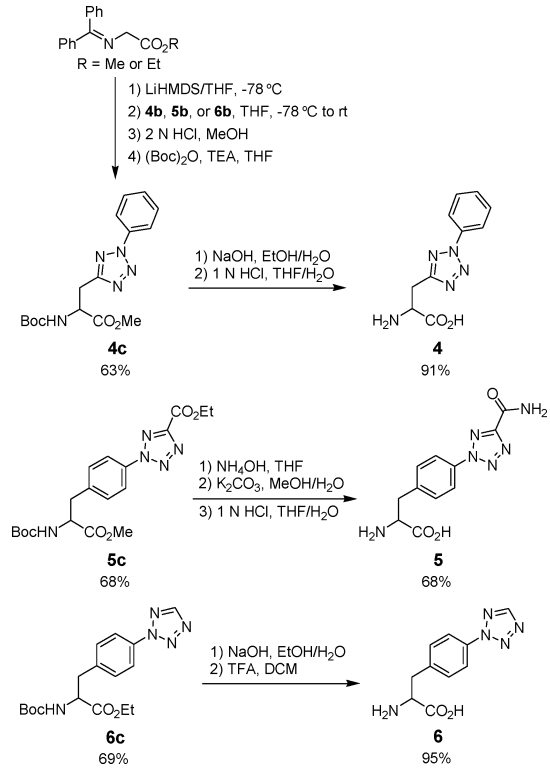
large aromatic side chains, including benzophenone, naphthyl, dansyl, 7-amino-coumarin, azobenzene, and bipyridyl groups, have been genetically incorporated into proteins,[14] the coplanar geometry of 2,5-diaryltetrazole[9b] in **1**−**3** may present a challenge to the biosynthetic enzymes involved in genetic incorporation of unnatural amino acids. To this end, we further designed several sterically less demanding monoaryltetrazole amino acids **4**−**6** with only two aryl rings (one phenyl and one tetrazole) at the side chain (Scheme 1). Our preliminary studies revealed that the N-aryl ring is absolutely required for the tetrazole photoreactivity.[15] As a result, we designed both type I (**4**) and type II (**5**, **6**) monoaryltetrazole amino acids and envisioned that all three of them can be readily accessed in racemic forms by alkylation of a glycine Schiff base with the requisite tetrazole-containing alkyl halides (Schemes 3 and 4).

Scheme 3. Synthesis of Tetrazole-Containing Alkyl Halides



Since the synthesis of 2-phenyl-5-carboxylatetetrazole has been reported in the literature,[16] we decided to employ this type of monoaryltetrazole to derive three tetrazole-containing alkyl halides (Scheme 3). Starting from ethyl glyoxalate, Kakehi's method rapidly gave rise to ethyl 2-phenyltetrazole-5-carboxylate (**4a**) and ethyl 2-tolyl-tetrazole-5-carboxylate (**5a**) in 60% and 41% yield, respectively. The type I tetrazole amino acid side chain, *N*-phenyltetrazole-5-methyl iodide (**4b**), was obtained by

Scheme 4. Synthesis of Monoaryl Tetrazole Amino Acids **4**−**6**



reducing the ester to alcohol followed by iodination with iodine and triphenylphosphine in the presence of imidazole for a combined 99% yield. For the type II tetrazole amino acid side chains, benzyl bromide **5b** was derived in 91% yield by treating **5a** with *N*-bromo succinimide (NBS) and benzoyl peroxide (BPO) in a straightfoward manner. Because simple 2-phenyltetrazole was shown to be photoreactive toward methyl acrylate in benzene,[17] we decided to remove the ester group in **5a** through decarboxylation[16] and obtained the second benzyl bromide **6b** in 49% yield by following an identical procedure as **5b** (Scheme 3).

The installation of the photoreactive tetrazole side chains to glycine Schiff base proceeded smoothly (Scheme 4). The Boc-protected methyl (or ethyl) esters of monoaryl tetrazole amino acids (**4c**, **5c**, and **6c**) were obtained in four steps with the key step involving the alkylation of glycine Schiff base with an excess amount of the appropriate tetrazole-containing alkyl halide (**4b**, **5b**, or **6b**) with overall yields of 63%, 68%, and 69%, respectively (Scheme 4). Simple removal of the protecting groups afforded monoaryl tetrazole amino acids **4** and **6** in excellent yields. It is noteworthy that amino acids **4** and **6** are structural isomers but potentially have discrete photoreactivities. To reduce side chain bulkiness and improve water solubility, we subjected the ethyl ester **5c**

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(15) In our initial study, 2-phenyl-5-ethyltetrazole reacted with methyl methacrylate to give rise to the pyrazoline adduct in 60% yield upon photoirradiation at 302 nm in ethyl acetate for 2 h, whereas analogous 2-ethyl-5-phenyltetrazole did not react under identical conditions: Wang, Y., Lin, Q. Unpublished results.

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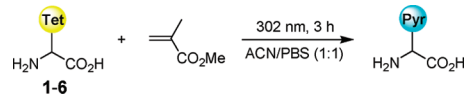
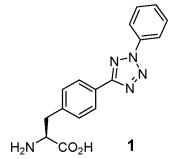
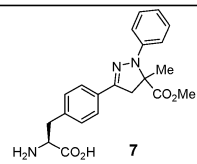
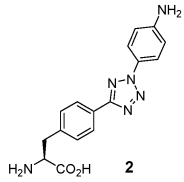
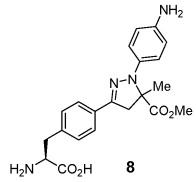
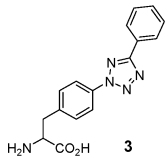
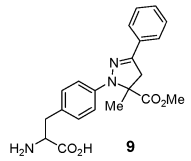
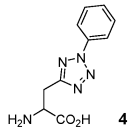
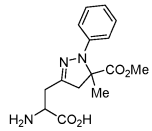
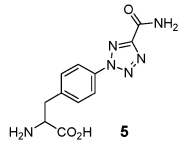
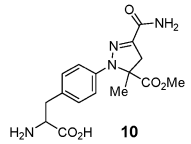
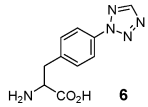
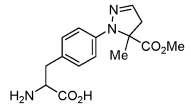
Table 1. Photoreactivity of Tetrazole Amino Acids **1**−**6** toward Methyl Methacrylate in Photoinduced 1,3-Dipolar Cycloaddition[a]



entry	tetrazole amino acid	product	yield[b]
1	 **1**	 **7**	99%[c]
2	 **2**	 **8**	98%
3	 **3**	 **9**	98%
4	 **4**		ND[d]
5	 **5**	 **10**	93%
6	 **6**		ND[d]

[a] Reactions were conducted by irradiating 0.06 mmol of **1**−**6** and 6 mmol of methyl methacrylate in 200 mL of ACN/PBS buffer (1:1) in quartz flasks for 3 h using a hand-held 302-nm UV lamp. pyr = pyrazoline. [b] Yields were determined by HPLC analysis. The standard deviation in yields should be less than 5% as the purified products at the indicated retention times (see Supporting Information) were greater than 95% pure on the basis of the ^{1}H NMR analysis. [c] 2 h reaction time. [d] No pyrazoline cycloadduct was detected.

to aminolysis and obtained 5-amido-2-phenyltetrazole amino acid **5** in 68% yield after deprotection (Scheme 4).

Having obtained these six tetrazole amino acids, we then examined their photoreactivities toward methyl methacrylate in a mixed ACN/PBS buffer (1:1) upon photoirradiation at 302 nm using a hand-held UV lamp (UVP, 302 nm, 0.16 A) (Table 1). We found that all three diaryltetrazole amino acids **1**−**3** reacted quantitatively with methyl methacrylate to afford the pyrazoline cycloadducts **7**−**9** based on the HPLC analysis. The reactions were highly regioselective[9] as the other regioisomers were not detected by either HPLC or NMR. However, among three monoaryltetrazole amino acids (**4**−**6**), only **5** gave rise to the pyrazoline cycloadduct in 93% yield with exclusive regioselectivity. While both **4** and **6** underwent the photoinduced tetrazole ring rupture (based on the disappearance of the starting materials), no pyrazoline cycloadducts were detected, presumably because of rapid water-quenching of the nonstabilized nitrile imine intermediates. By contrast, because of the resonance effect, tetrazole amino acids **1**−**3** and **5** generate much more stable nitrile imine dipoles in the aqueous buffer, *e.g.*, a diaryltetrazole-derived nitrile imine intermediate was detected by reverse-phase HPLC and showed a half-life of 5 s.[10a]

In summary, we report the first synthesis of six unique tetrazole amino acids, four of which showed excellent reactivity toward a simple alkene in the photoinduced cycloaddition reaction in biological buffer. The incorporation of these photoreactive amino acids site-specifically into proteins to serve as chemical models for protein post-translational modifications, such as lipidation and phosphorylation, is currently underway.

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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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