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Synthesis of Macrocyclic Tetrazoles for Rapid Photoinduced Bioorthogonal 1,3-Dipolar Cycloaddition Reactions

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Abstract

A series of conformationally constrained, macrocyclic tetrazoles were expediently prepared by double alkylation of a bis(*o*-phenolyl)tetrazole precursor. Several of them showed excellent reactivity toward norbornene in a photoinduced tetrazole-alkene cycloaddition reaction in organic solvent as well as toward a norbornene-modified protein in PBS buffer.

Keywords

bioorthogonal chemistry; tetrazole; alkene; cycloaddition; biotechnology

There is increasing interest to develop robust bioorthogonal reactions for site-specific modification of biomolecules within living systems.[1] A prominent example is the copper-catalyzed azide-alkyne cycloaddition reaction ("CuAAC").[2] Since copper is toxic to cells, there have been substantial efforts directed toward developing copper-free alternatives for applications in live cells. One strategy involves activation of dipolarophiles by constraining them in macrocyclic rings: several cyclooctynes have been developed for rapid 1,3-dipolar cycloadditions with azide.[3] However, the use of ring structures containing strained dipoles for rapid bioorthogonal 1,3-dipolar cycloaddition reactions was extremely rare,[4] despite the fact that many cyclic dipoles have been used successfully in organic synthesis, e.g., cyclic azomethine imine,[5] cyclic carbonyl ylide,[6] cyclic azomethine ylide,[7] and cyclic nitrones[8] (Scheme 1). To our knowledge, cyclic nitrile imines—an important class of 1,3-dipoles[9] for the preparation of pyrazolines—has not been reported in the literature.

We have recently reported the use of diaryltetrazoles as photoactivatable precursors to the reactive nitrile imine dipoles for a photoinduced, bioorthogonal cycloaddition reaction with both the electron-deficient alkenes[10] and the unactivated terminal alkenes.[11] In a photocrystallographic study, we found that upon photoirradiation a diaryltetrazole generated *in situ* a bent nitrile imine,[12] the geometry postulated to exhibit reduced distortion energy and thus higher reactivity based on recent computational studies.[13] To reinforce the nitrile imine geometry in this reactive conformation, we hypothesized that installing a short "bridge" between the *ortho*-positions of two flanking phenyl rings to form a macrocyclic diphenyltetrazole should generate a cyclic nitrile imine upon photoirradiation with an overall reduced rotational freedom. Toward this goal, here we report the synthesis of a series of conformationally constrained macrocyclic tetrazoles and the characterization of their reactivity toward both a terminal alkene and a strained alkene in organic solvents as well as a norbornene-modified lysozyme in phosphate buffered saline (PBS).

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To create the attachment sites for the installation of a chemical "bridge", we synthesized two linear tetrazoles, 2,5-bis(*o*-phenolyl)-tetrazole (**1**) and 2-*o*-phenolyl-5-o-anilinyltetrazole (**7**) using the Kakehi method.[14] To effect macrocyclization via bis-alkylation, we treated tetrazole **1** with 1.1 equiv of dibromoalkanes with variable carbon length and screened four alkali metal bases: LiOH, Na_2CO_3, K_2CO_3 and Cs_2CO_3, anticipating that alkali metal ions with the right size may facilitate ring closure by bringing two phenol-O to the N1-side of tetrazole **1** through chelation (Table 1). Gratifyingly, we found that bases with larger alkali metals such as Cs^+ and K^+ in general afforded higher yields of the desired macrocyclic products (entries 7–8, 11–12, 15–16, and 19–20). Yields were lowest for tetrazole **2** (entries 3–4), presumably due to its smallest ring size (12-membered ring). Li^+ and Na^+ bases generally produced only trace amounts of the desired macrocyclic products together with large amounts of intractable oligomers. This metal ion size-dependency suggests that the larger ions Cs^+ and K^+ may form more stable, tridentate chelates with tetrazole **1** than the smaller ions. We then designed tetrazole **9** (Table 1), suspecting that amido-H may form an internal hydrogen bond with tetrazole-N1 which should reinforce the bent nitrile imine geometry. Thus, tetrazole **9** was readily prepared through amidation of tetrazole **7** with 4-chloro-butanoyl chloride followed by an intramolecular *O*-alkylation. Notably, Na_2CO_3 was found to be the optimal base for the ring closure reaction (compare entries 21–24).

The structures of macrocyclic tetrazoles **6** and **9** were confirmed by X-ray crystallography (Figure 1). To our surprise, the amide bond in **9** was engaged in a pair of intermolecular H-bonds between N5-H and N1 of the neighboring tetrazole instead of an internal one. The three conjoining aromatic rings are slightly twisted out of the tetrazole plane with the torsional angles of N1-N2-C2-C3 and N1-C1-C8-C13 to be 45.12° and −44.38°, respectively (for tetrazole **6**), and −62.46° and 37.17°, respectively (for tetrazole **9**). These distortions might be due to lone-pair repulsion between N1 and O1 (distance: 2.815 Å for **6**, 2.848 Å for **9**) and between N1 and O2 (distance: 2.857 Å for **6**, 3.249 Å for **9**).

To assess the reactivity of macrocyclic tetrazoles, we carried out the photoinduced cycloaddition reactions with either 4-penten-1-ol, an analogue of a bioorthogonal alkene reporter homoallylglycine,[15] or a strained alkene reporter norbornene[16] in ethyl acetate (Table 2). Because the reaction proceeds through two distinct steps: ring opening step to generate the cyclic nitrile imine in situ and the subsequent [3+2] cycloaddition reaction,[10] we monitored the reactions by thin layer chromatography and discontinued the reactions once the tetrazole starting materials were consumed. We found that in general macrocyclic tetrazoles gave higher yields of the pyrazoline cycloadducts (with exceptions of entries 5, 6, 11) compared to their acyclic counterparts (entries 7, 14). As expected, norbornene serves as a more reactive dipolarophile as compared with 4-penten-1-ol, affording the corresponding pyrazolines in higher yields (compare entries 8–14 to 1–7). Among the macrocyclic tetrazoles, the 3- and 4-carbon linked tetrazoles **2** and **3** showed markedly higher reactivity with shorter reaction times and higher yields (entries 2, 8, 9), presumably due to their smaller ring sizes and thus reduced conformational flexibility. It is interesting to note that *O*-xylylene-linked tetrazole **6** gave a moderate yield with 4-penten-1-ol (entry 5) but a good yield with norbornene (entry 12), which can be attributed to a com-pact exo TS stabilized by attractive edge-to-face C—H•••π interaction between norbornene bridge C—H and xylyl-π crowd.[17] Gratifyingly, the X-ray structure of pyrazoline **3b** was obtained, showing that the two phenyl rings are mostly co-planar with the pyrazoline ring with the torsional angles of N1-N2-C15-C16 and N1-C1-C9-C14 to be 30.8° and −26.6°, respectively (Figure 2).

The excellent reactivity of the macrocyclic tetrazoles toward norbornene prompted us to measure the photophysical properties of the resulting macrocyclic pyrazolines (Table 3). Relative to the pyrazoline control **10b** (entry 7), four macrocyclic pyrazolines showed the bathochromic shifts in λ_{abs} (entries 1–2, 5–6) while two pyrazolines showed the

hypsochromic shifts (entries 3–4), presumably due to their twisted macrocyclic geometries. Compared to **10b**, macrocyclic pyrazolines exhibited invariable hypsochromic shifts in the maximum emission wavelength (λ_{em}) along with increases in the fluorescence quantum yield (φ_F).

To examine whether the macrocyclic tetrazoles can be used to label a norbornene-containing protein, we incubated tetrazoles **2**–**6** (200 μM) with a norbornene-modified lysozyme (10 μM) in PBS buffer. The mixtures were photoirradiated with a handheld 302-nm UV lamp for 1 min prior to quenching with SDS sample buffer. In-gel fluorescence analysis (Figure 3) indicated that tetrazoles **2**, **3** and **6**, along with acyclic tetrazole **10**, showed specific pyrazoline cycloadducts, in good agreement with their generally higher reactivity (entries 8, 9 and 12 in Table 2) and their superior photophysical properties (entries 1, 2 and 5 in Table 3). In particular, tetrazole **6** showed the strongest fluorescent band, presumably due to rate enhancement in water as a result of tighter hydrophobic packing in the TS. However, initial efforts to determine the reaction yields using mass spectrometry were unsuccessful due to the poor solubility of the pyrazoline-cycloadducts in PBS buffer.

In summary, we have synthesized a series of structurally novel macrocyclic diphenyl tetrazoles by installing a short “bridge” between the two flanking diphenyl rings. Compared to the acyclic tetrazole, several macrocyclic tetrazoles showed improved reactivity toward a strained alkene both in organic solvent. One macrocyclic tetrazole showed rapid (~ 1 min) functionalization of a norbornene-modified protein in PBS buffer. These macrocyclic tetrazoles should offer a new class of photoactivatable tetrazole reagents for the bioorthogonal tetrazole-alkene cycloaddition reaction in living systems.

Experimental Section

Photoinduced Cycloaddition of Lyso-Norbornene with Macrocyclic Tetrazoles: To a 20-μL solution of either Lyso or Lyso-Nor (10 μM) in PBS buffer, pH 7.4, in a 96-well microtiter plate was added 1 μL of the various tetrazoles (4 mM in DMSO, final concentration is 200 μM). The mixtures were irradiated with a handheld 302-nm UV lamp for 1 min before quenching by addition of 5 μL of 6× SDS sample buffer, boiled for 5 min at 95 °C, loaded onto a NuPAGE 12% Bis-Tris gel (Invitrogen), and then subjected to protein electrophoresis. The Lyso-Pyr cycloadducts in the gel were visualized by illuminating the gel with a handheld 365-nm UV lamp and the resulting image was captured by a digital camera. After the image acquisition, the same gel was stained with Coomassie blue to confirm the size and equal loading of proteins.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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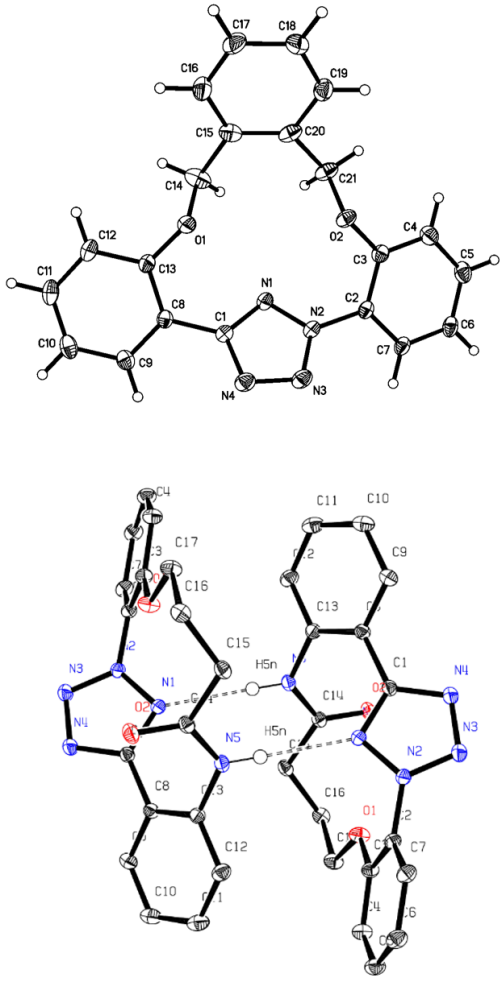


Figure 1.
ORTEP diagrams of macrocyclic tetrazole **6** (top) and the dimer of macrocyclic tetrazole **9** (bottom) shown at 50% probability.

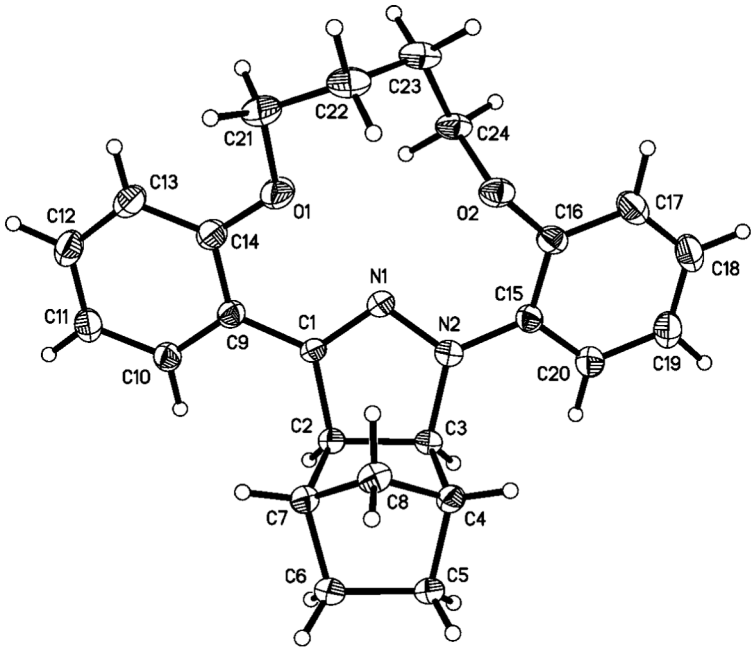


Figure 2.
ORTEP diagram of the macrocyclic pyrazoline **3b** shown at 50% probability.

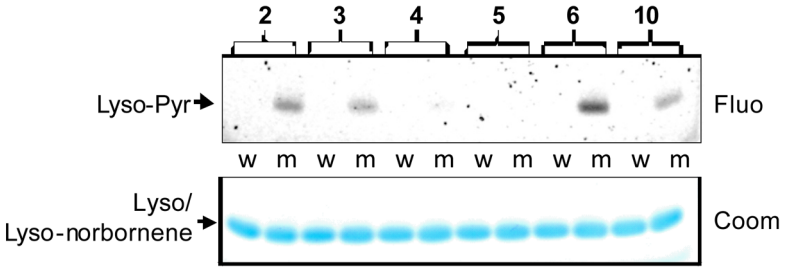
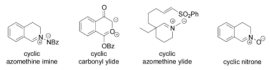


Figure 3.

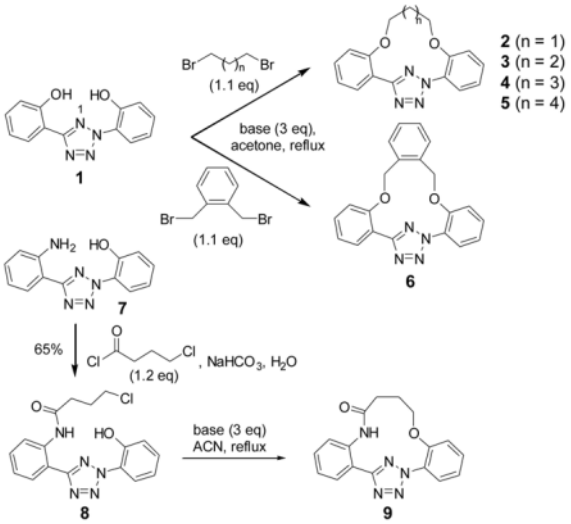
Photoinduced cycloaddition reaction of tetrazoles with a norbornene-modified lysozyme (m) or wild-type lysozyme (w) at 302 nm: top panel, inverted in-gel fluorescence with λ_{ex} = 365 nm; bottom panel, Coomassie blue staining.



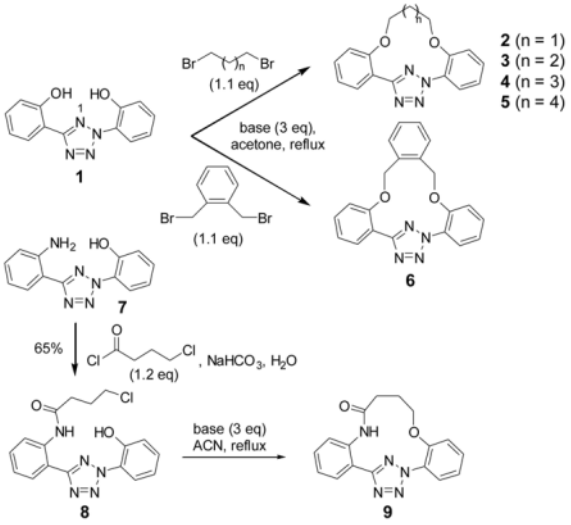
Scheme 1.
Representative cyclic 1,3-dipoles.

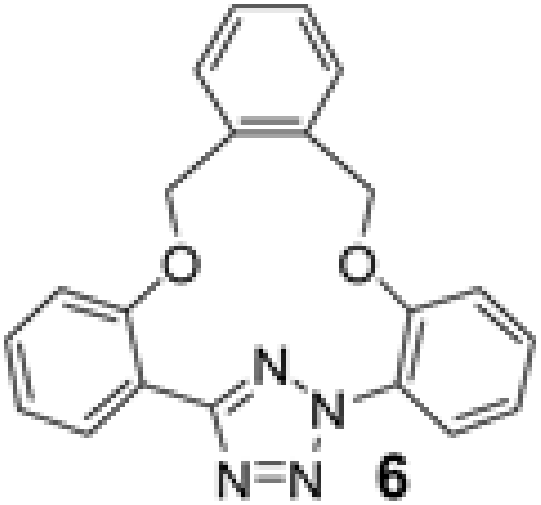
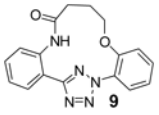
Table 1

Synthesis of the macrocyclic tetrazoles[a].



entry	base	product	yield (%)[b]
1	$LiOH•H_2O$		trace
2	Na_2CO_3		<5[c]
3	K_2CO_3		<30[c]
4	Cs_2CO_3		38
5	$LiOH•H_2O$		trace
6	Na_2CO_3		trace
7	K_2CO_3		51
8	Cs_2CO_3		78
9	$LiOH•H_2O$		trace
10	Na_2CO_3		trace
11	K_2CO_3		<35[c]
12	Cs_2CO_3		45
13	$LiOH•H_2O$		trace
14	Na_2CO_3		trace
15	K_2CO_3		<60[c]
16	Cs_2CO_3		67



entry	base	product	yield (%)[b]
17	$LiOH•H_2O$		trace
18	Na_2CO_3		trace
19	K_2CO_3		<50[c]
20	Cs_2CO_3		61
21	$LiOH•H_2O$		trace
22	Na_2CO_3		70
23	K_2CO_3		<20[c]
24	Cs_2CO_3		trace

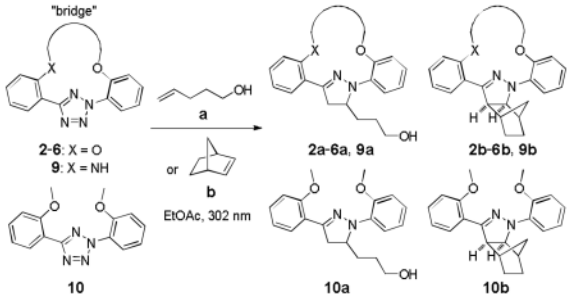
[a] For bis-O-alkylation, reactions were carried out by refluxing tetrazole **1** with 1.1 equiv of dibromide and 3 equiv of base in acetone overnight. For intramolecular *O*-alkylation, tetrazole **8** was refluxed in acetonitrile with 3 equiv of base.

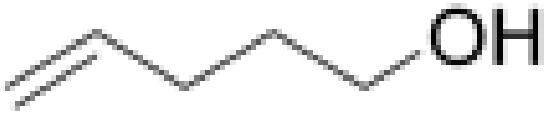
[b] Isolated yields were reported unless noted otherwise.

[c] Estimated yields based on TLC.

Table 2

Photoinduced 1,3-Dipolar Cycloaddition Reactions of Macrocyclic and Acylic Tetrazoles with Alkenes[a].



entry	alkene	tetrazole	pyrazoline	time[b] (min)	yield[c] (%)
1		**2**	**2a**	120	59
2		**3**	**3a**	75	71
3		**4**	**4a**	84	60
4		**5**	**5a**	180	70
5		**6**	**6a**	120	46[d]
6	**a**	**9**	**9a**	300	43
7		**10**	**10a**	120	58
8		**2**	**2b**	108	95
9		**3**	**3b**	54	91
10		**4**	**4b**	120	81
11		**5**	**5b**	240	60
12		**6**	**6b**	90	84
13	**b**	**9**	**9b**	120	84
14		**10**	**10b**	60	76

[a]Reactions were conducted by irradiating 0.1 mmol of tetrazole and 10 mmol of alkene dipolarophile in 250 mL EtOAc with a 302-nm UV lamp in quartz flask.

[b]Time was determined by tracing the disappearance of the starting materials on TLC.

[c]Isolated yields.

[d]The pyrazoline adduct was unstable upon standing.

Table 3

Photophysical properties of macrocyclic pyrazolines[a].

entry	pyrazoline	λ_{abs} (nm)	ε (M^{-1} cm^{-1})	λ_{em} (nm)[b]	φ_F[c]
1	**2b**	358	20,356	456	0.30
2	**3b**	364	10,190	466	0.35
3	**4b**[d]	348	1,702	468	0.35
4	**5b**[d]	348	830	464	0.17
5	**6b**	358	13,852	451	0.32
6	**9b**	376	19,440	477	0.25
7	**10b**	350	12,534	502	0.15

[a] Compounds were dissolved in PBS:ACN (1:1) at the concentrations of 5 μM unless noted otherwise.

[b] λ_{ex} = 348 nm.

[c] Quantum yields were determined using DAPI as the standard (φ_F = 0.58 in DMSO).

[d] 20 μM concentrations were used.

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