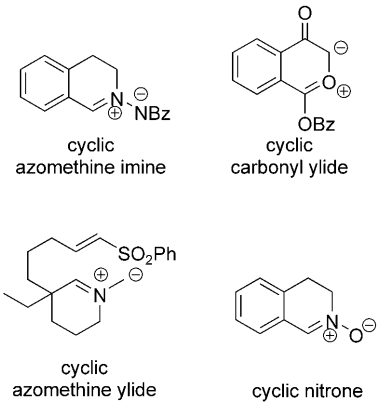
DOI: 10.1002/chem.201002360

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

Zhipeng Yu, Reyna K. V. Lim, and Qing Lin*[a]

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 living 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 is extremely rare,[4] despite the fact that many cyclic dipoles have been used successfully in organic synthesis, for example, cyclic azomethine imine,[5] cyclic carbonyl ylide,[6] cyclic azomethine ylide,[7] and cyclic nitrones[8] (Scheme 1). To the best of our knowledge, cyclic nitrile imines—a class of 1,3-dipoles[9] important for the preparation of pyrazolines—have not been reported in the literature.

We recently reported the use of diaryltetrazoles as photoactivatable precursors to the reactive nitrile imine dipoles for photoinduced, bioorthogonal cycloaddition reactions with both electron-deficient alkenes[10] and unactivated terminal alkenes.[11] In a photocrystallographic study, we found that a bent nitrile imine was generated in situ upon photoirradiation of a diaryltetrazole.[12] The bent geometry is 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 inserting a short bridge between the *ortho* positions of the two flanking phenyl rings to form a macrocyclic diphenyltetrazole should, upon photoirradiation, generate a cyclic nitrile imine with an overall reduced rotational freedom. Herein 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).



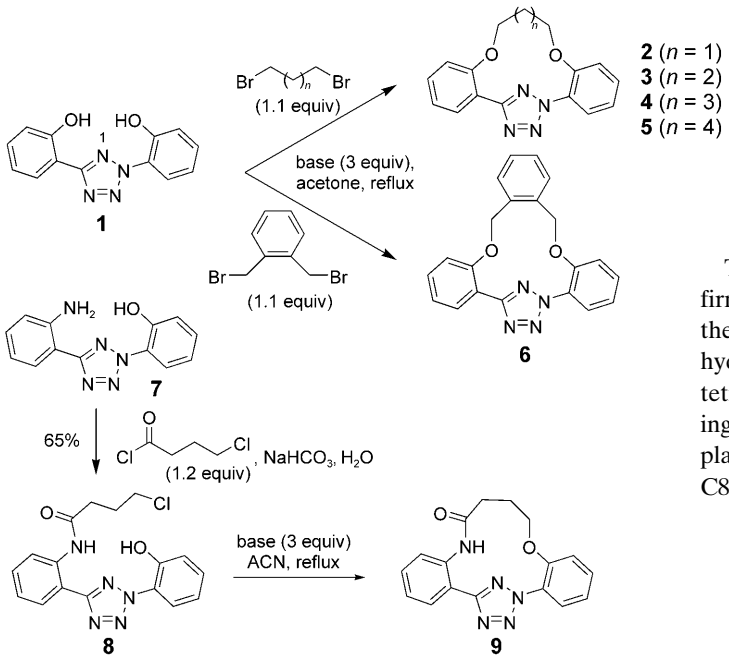
Scheme 1. Representative cyclic 1,3-dipoles.

To create the attachment sites for 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] For macrocyclization by bis-alkylation, we treated tetrazole **1** with dibromoalkanes (1.1 equiv) with variable lengths of the carbon chain 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 the two phenol O atoms to the N1 side of tetrazole **1** together 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 (Table 1, entries 7, 8, 11, 12, 15, 16, 19, and 20); tetrazole **2** (Table 1, entries 3 and 4) had the lowest yields, presumably due to the small ring size (12-membered ring). Li^+ and Na^+ bases generally produced only trace amounts of the desired macrocyclic products to-

[a] Dr. Z. Yu, R. K. V. Lim, Prof. Dr. Q. Lin
Department of Chemistry
State University of New York at Buffalo
Buffalo, NY 14260 (USA)
Fax: (+1) 716-645-6963
E-mail: qinglin@buffalo.edu

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201002360.

Table 1. Synthesis of the macrocyclic tetrazoles.[a]



Entry	Base	Product	Yield [%][b]
1	$LiOH·H_2O$	**2**	trace
2	Na_2CO_3	**2**	<5[c]
3	K_2CO_3	**2**	<30[c]
4	Cs_2CO_3	**2**	38
5	$LiOH·H_2O$	**3**	trace
6	Na_2CO_3	**3**	trace
7	K_2CO_3	**3**	51
8	Cs_2CO_3	**3**	78
9	$LiOH·H_2O$	**4**	trace
10	Na_2CO_3	**4**	trace
11	K_2CO_3	**4**	<35[c]
12	Cs_2CO_3	**4**	45
13	$LiOH·H_2O$	**5**	trace
14	Na_2CO_3	**5**	trace
15	K_2CO_3	**5**	<60[c]
16	Cs_2CO_3	**5**	67
17	$LiOH·H_2O$	**6**	trace
18	Na_2CO_3	**6**	trace
19	K_2CO_3	**6**	<50[c]
20	Cs_2CO_3	**6**	61
21	$LiOH·H_2O$	**9**	trace
22	Na_2CO_3	**9**	70
23	K_2CO_3	**9**	<20[c]
24	Cs_2CO_3	**9**	trace

[a] For bis-O-alkylation, reactions were carried out by heating tetrazole **1** at reflux with dibromide (1.1 equiv) and base (3 equiv) in acetone overnight. For intramolecular O-alkylation, tetrazole **8** was heated at reflux in acetonitrile with base (3 equiv). [b] Isolated yields were reported unless noted otherwise. [c] Estimated yields based on TLC.

gether 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 with the smaller ions. We then designed tetrazole **9** (Table 1), suspecting that the amido-H may form an internal hydrogen bond with tetrazole-N1, which would reinforce the bent nitrile imine geometry. Thus, tetrazole **9** was readily prepared through amidation of tetrazole **7** with 4-chlorobutanoyl 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 in Table 1).

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 hydrogen bonds between N5–H and N1 of the neighboring tetrazole instead of internal interactions. The three conjoining aromatic rings are slightly twisted out of the tetrazole plane with the torsional angles N1-N2-C2-C3 and N1-C1-C8-C13 of 45.12 and −44.38°, respectively (for tetrazole **6**),

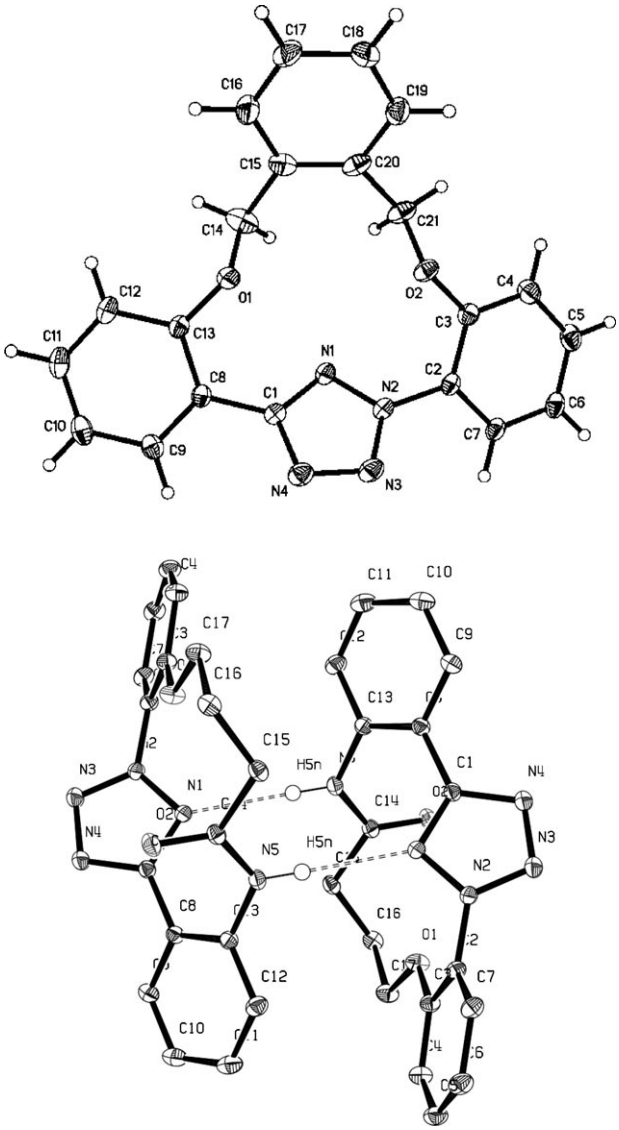


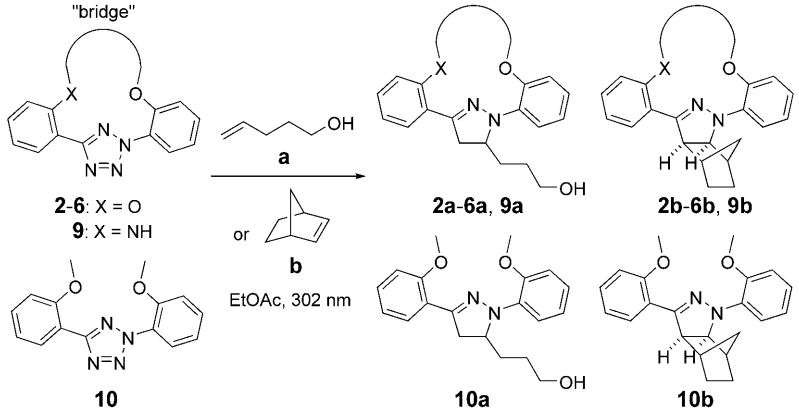
Figure 1. ORTEP diagrams of macrocyclic tetrazole **6** (top) and the dimer of macrocyclic tetrazole **9** (bottom) shown at 50% probability.

and −62.46 and 37.17°, respectively (for tetrazole **9**). These distortions might be due to lone-pair repulsions 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 the macrocyclic tetrazoles, we carried out 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, a 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 (TLC) and discontinued the reactions once the tetrazole starting materials were consumed. The macrocyclic tetrazoles gave, in general, higher yields of the pyrazoline cycloadducts (with the exceptions of entries 5, 6, and 11 in Table 2) compared to their acyclic counterparts (Table 2, entries 7 and 14). As expected, norbornene serves as a more reactive dipolarophile than 4-penten-1-ol, affording the corresponding pyrazolines in higher yields (compare entries 8–14 with 1–7, Table 2). Among the macrocyclic tetrazoles, the three- and four-carbon-linked tetrazoles **2** and **3** showed markedly higher reactivity with shorter reaction times and higher yields (Table 2, entries 2, 8, and 9), presumably due to the smaller ring sizes and thus reduced conformational flexibility. Interestingly, the *O*-xylylene-linked tetrazole **6** gave a moderate yield with 4-penten-1-ol (Table 2, entry 5), but a good yield with norbornene (Table 2, entry 12), which can be attributed to a compact *exo* transition state (TS) stabilized by the attractive edge-to-face C–H···π interaction between the norbornene bridge C–H and the xylyl π electrons.[17] Gratifyingly, the X-ray structure of pyrazoline **3b** was obtained, showing that the two phenyl rings are almost coplanar with the pyrazoline ring with torsional angles N1-N2-C15-C16 and N1-C1-C9-C14 of 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). Four macrocyclic pyrazolines showed bathochromic shifts in λ_{abs} (Table 3, entries 1, 2, 5, and 6) relative to the pyrazoline control **10b** (Table 3, entry 7), while two pyrazolines showed the hypsochromic shifts (Table 3, entries 3

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	**a**	**2**	**2a**	120	59
2	**a**	**3**	**3a**	75	71
3	**a**	**4**	**4a**	84	60
4	**a**	**5**	**5a**	180	70
5	**a**	**6**	**6a**	120	46[d]
6	**a**	**9**	**9a**	300	43
7	**a**	**10**	**10a**	120	58
8	**b**	**2**	**2b**	108	95
9	**b**	**3**	**3b**	54	91
10	**b**	**4**	**4b**	120	81
11	**b**	**5**	**5b**	240	60
12	**b**	**6**	**6b**	90	84
13	**b**	**9**	**9b**	120	84
14	**b**	**10**	**10b**	60	76

[a] Tetrazole (0.1 mmol) and alkene dipolarophile (10 mmol) in EtOAc (250 mL) were irradiated 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.

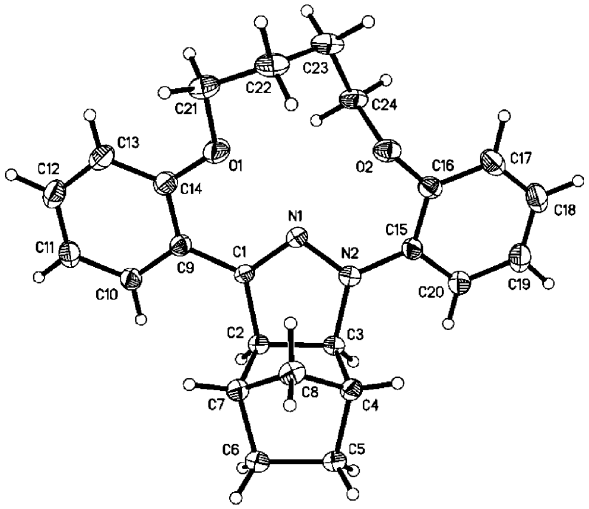


Figure 2. ORTEP diagram of the macrocyclic pyrazoline **3b** shown at 50% probability.

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	20356	456	0.30
2	**3b**	364	10190	466	0.35
3	**4b**[d]	348	1702	468	0.35
4	**5b**[d]	348	830	464	0.17
5	**6b**	358	13852	451	0.32
6	**9b**	376	19440	477	0.25
7	**10b**	350	12534	502	0.15

[a] Compounds were dissolved in PBS/CH_3CN (1:1) at concentrations of 5 μM unless noted otherwise. [b] λ_{ex} = 348 nm. [c] Quantum yields were determined by using 4′,6-diamino-2-phenylindole (DAPI) as the standard (Φ_F = 0.58 in DMSO). [d] 20 μM concentrations were used.

and 4), presumably due to their twisted macrocyclic geometries. Compared with **10b**, the 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 sodium dodecyl sulfate (SDS) sample buffer. In-gel fluorescence analysis (Figure 3) indicated specific pyr-

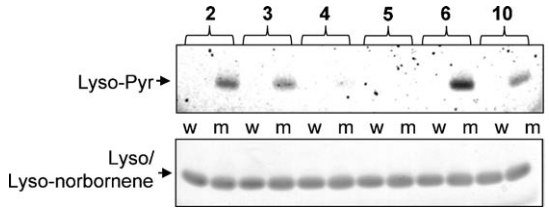


Figure 3. Photoinduced cycloaddition reaction of tetrazoles with a norbornene-modified lysozyme (m) or wild-type lysozyme (w) at 302 nm. The top panel represents the inverted in-gel fluorescence with λ_{ex} = 365 nm, and bottom panel the Coomassie blue staining.

azoline cycloadducts for tetrazoles **2**, **3**, and **6**, along with acyclic tetrazole **10**, agreeing well 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). 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 by 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 inserting a bridge between the two flanking diphenyl rings. Compared with the acyclic tetrazole, several macrocyclic tetrazoles showed improved reactivity toward a strained alkene 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: Various tetrazoles (1 μL; 4 mM in DMSO, to a final concentration of 200 μM) were added to a 20 μL solution of either lysozyme (lyso) or lyso-norbornene (10 μM) in PBS buffer, pH 7.4, in a 96-well microtiter plate. The mixtures were irradiated with a handheld 302 nm UV lamp for 1 min before quenching by addition of 6×SDS (5 μL) 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 image acquisition, the same gel was stained with Coomassie blue to confirm the size and equal loading of proteins.

CCDC-783273 (**6**), 783275 (**9**), and 783274 (**3b**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Acknowledgements

We gratefully acknowledge the National Institutes of Health (GM 85092) for financial support. We thank William Brennessel at University of Rochester for X-ray structural determination.

Keywords: alkenes · bioorthogonal chemistry · biotechnology · cycloaddition · tetrazoles

[1] a) E. M. Sletten, C. R. Bertozzi, *Angew. Chem.* **2009**, *121*, 7108–7133; *Angew. Chem. Int. Ed.* **2009**, *48*, 6974–6998; b) R. K. V. Lim, Q. Lin, *Chem. Commun.* **2010**, *46*, 1589–1600; c) J. C. Jewett, C. R. Bertozzi, *Chem. Soc. Rev.* **2010**, *39*, 1272–1279.

[2] a) V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem.* **2002**, *114*, 2708–2711; *Angew. Chem. Int. Ed.* **2002**, *41*, 2596–2599; b) C. W. Tornoe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057–3064.

[3] a) For an excellent review, see: M. F. Debets, C. W. van der Doelen, F. P. Rutjes, F. L. van Delft, *ChemBioChem* **2010**, *11*, 1168–1184; b) N. J. Agard, J. A. Prescher, C. R. Bertozzi, *J. Am. Chem. Soc.* **2004**, *126*, 15046–15047; c) N. J. Agard, J. M. Baskin, J. A. Prescher, A. Lo, C. R. Bertozzi, *ACS Chem. Biol.* **2006**, *1*, 644–648; d) J. M. Baskin, J. A. Prescher, S. T. Laughlin, N. J. Agard, P. V. Chang, I. A. Miller, A. Lo, J. A. Codelli, C. R. Bertozzi, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 16793–16797; e) X. Ning, J. Guo, M. A. Wolfert, G. J. Boons, *Angew. Chem.* **2008**, *120*, 2285–2287; *Angew. Chem. Int. Ed.* **2008**, *47*, 2253–2255; f) M. F. Debets, S. S. van Berkel, S. Schoffelen, F. P. J. T. Rutjes, J. C. M. van Hest, F. L. van Delft, *Chem. Commun.* **2010**, *46*, 97–99; g) J. C. Jewett, E. M. Sletten, C. R. Bertozzi, *J. Am. Chem. Soc.* **2010**, *132*, 3688–3690.

[4] C. S. McKay, J. Moran, J. P. Pezacki, *Chem. Commun.* **2010**, *46*, 931–933.

[5] a) H. Dorn, A. Otto, *Angew. Chem.* **1968**, *80*, 196–196; *Angew. Chem. Int. Ed. Engl.* **1968**, *7*, 214–215; b) W. E. Truce, J. R. Allison, *J. Org. Chem.* **1975**, *40*, 2260–2261; c) T. Hashimoto, Y. Maeda, M. Omote, H. Nakatsu, K. Maruoka, *J. Am. Chem. Soc.* **2010**, *132*, 4076–4077.

[6] a) A. Padwa, S. P. Carter, H. Nimmesgern, *J. Org. Chem.* **1986**, *51*, 1157–1158; b) A. Padwa, S. P. Carter, H. Nimmesgern, P. D. Stull, *J.*

Am. Chem. Soc. **1988**, *110*, 2894–2900; c) A. Padwa, G. E. Fryxell, L. Zhi, *J. Am. Chem. Soc.* **1990**, *112*, 3100–3109.

[7] a) G. Pandey, T. D. Bagul, A. K. Sahoo, *J. Org. Chem.* **1998**, *63*, 760–768; b) W. H. Pearson, P. Stoy, Y. Mi, *J. Org. Chem.* **2004**, *69*, 1919–1939; c) I. Coldham, A. J. M. Burrell, L. E. White, H. Adams, N. Oram, *Angew. Chem.* **2007**, *119*, 6271–6274; *Angew. Chem. Int. Ed.* **2007**, *46*, 6159–6162; d) I. Coldham, S. Jana, L. Watson, C. D. Pilgram, *Tetrahedron Lett.* **2008**, *49*, 5408–5410.

[8] a) For a recent review, see: J. Revuelta, S. Cicchi, A. Goti, A. Brandi, *Synthesis* **2007**, 485–504; b) F. Cardona, S. Valenza, S. Picasso, A. Goti, A. Brandi, *J. Org. Chem.* **1998**, *63*, 7311–7318; c) M. Thiverny, C. Philouze, P. Y. Chavant, V. Blandin, *Org. Biomol. Chem.* **2010**, *8*, 864–872; d) G. Ghini, L. Luconi, A. Rossin, C. Bianchini, G. Giambastiani, S. Cicchi, L. Lascialfari, A. Brandi, A. Giannasi, *Chem. Commun.* **2010**, *46*, 252–254.

[9] a) R. Huisgen, *Angew. Chem.* **1963**, *75*, 604–637; *Angew. Chem. Int. Ed. Engl.* **1963**, *2*, 565–598; b) R. Huisgen, *Angew. Chem.* **1963**, *75*, 742–754; *Angew. Chem. Int. Ed. Engl.* **1963**, *2*, 633–645; c) G. Bianchi, R. Gandolfi, *1,3-Dipolar Cycloaddition Chemistry* (A. Padwa), Wiley, New York, **1984**, pp. 470–477.

[10] a) Y. Wang, C. R. Vera, Q. Lin, *Org. Lett.* **2007**, *9*, 4155–4158; b) W. Song, Y. Wang, J. Qu, M. M. Madden, Q. Lin, *Angew. Chem.* **2008**, *120*, 2874–2877; *Angew. Chem. Int. Ed.* **2008**, *47*, 2832–2835; c) Y. Wang, W. J. Hu, W. Song, R. K. V. Lim, Q. Lin, *Org. Lett.* **2008**, *10*, 3725–3728; d) W. Song, Z. Yu, M. M. Madden, Q. Lin, *Mol. BioSyst.* **2010**, *6*, 1576–1578.

[11] a) W. Song, Y. Wang, J. Qu, Q. Lin, *J. Am. Chem. Soc.* **2008**, *130*, 9654–9655; b) Y. Wang, W. Song, W. J. Hu, Q. Lin, *Angew. Chem.* **2009**, *121*, 5434–5437; *Angew. Chem. Int. Ed.* **2009**, *48*, 5330–5333.

[12] S. L. Zheng, Y. Wang, Z. Yu, Q. Lin, P. Coppens, *J. Am. Chem. Soc.* **2009**, *131*, 18036–18037.

[13] a) D. H. Ess, K. N. Houk, *J. Am. Chem. Soc.* **2007**, *129*, 10646–10647; b) D. H. Ess, K. N. Houk, *J. Am. Chem. Soc.* **2008**, *130*, 10187–10198; c) F. Schoenebeck, D. H. Ess, G. O. Jones, K. N. Houk, *J. Am. Chem. Soc.* **2009**, *131*, 8121–8133.

[14] S. Ito, Y. Tanaka, A. Kakehi, K. Kondo, *Bull. Chem. Soc. Jpn.* **1976**, *49*, 1920–1923.

[15] W. Song, Y. Wang, Z. Yu, C. I. Rivera Vera, J. Qu, Q. Lin, *ACS Chem. Biol.* **2010**, *5*, 875–885.

[16] a) N. K. Devaraj, R. Weissleder, S. A. Hilderbrand, *Bioconjugate Chem.* **2008**, *19*, 2297–2299; b) K. Gutsmiedl, C. T. Wirges, V. Ehmke, T. Carell, *Org. Lett.* **2009**, *11*, 2405–2408; c) H. S. Han, N. K. Devaraj, J. Lee, S. A. Hilderbrand, R. Weissleder, M. G. Bawendi, *J. Am. Chem. Soc.* **2010**, *132*, 7838–7839.

[17] S. Paliwal, S. Geib, C. S. Wilcox, *J. Am. Chem. Soc.* **1994**, *116*, 4497–4498.

Received: August 17, 2010
Published online: October 28, 2010

