ORGANIC LETTERS

2012
Vol. 14, No. 10
2590–2593

Chemoselective Sequential "Click" Ligation Using Unsymmetrical Bisazides

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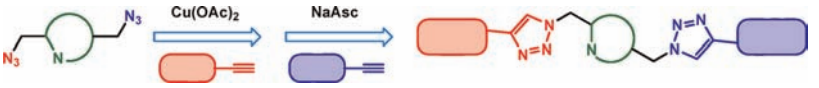
Received April 7, 2012

ABSTRACT



Unsymmetrical bisazides containing chelating and nonchelating azido groups undergo chemoselective three-component copper(I)-catalyzed azide−alkyne conjugation reactions with two different alkyne molecules. In conjunction with the reactivity gap between aromatic and aliphatic alkynes, a bistriazole molecule can be generated with excellent regioselectivity by mixing two alkynes and a bisazide in a single reaction container. This method is applicable in aqueous solutions at neutral pH, which may lend utilities in bioconjugation applications.

Chemoselective sequential ligation has been employed in applications including multiple labeling[1] and syntheses of macromolecular assemblages.[2] The two strategies in devising a sequential ligation are (1) successive coupling reactions of the same type that require the deprotection of the subsequent reactive site,[1a,2d,e] and (2) the utilization of two or more completely different reactions.[1b,c,2a−c] Recent research in bifunctional molecular linkers highlights the key challenge in developing chemoselective sequential ligation protocols, which is to achieve complete regiochemical control of the coupling reactions in a timely and cost-effective manner, while providing broad functional group tolerance.

The copper(I)-catalyzed azide−alkyne cycloaddition (CuAAC)[3] and its metal-free variants,[4] which are the most widely applied "click" reactions,[5] appear to be the methods of choice in developing bifunctional molecular linkers, due to their enormous substrate scopes and rapid reaction kinetics. Several examples of AAC-based bifunctional linkers are listed in Figure 1, in which differentiating the reactivities of alkyne substrates leads to chemoselectivity in sequential ligation reactions. Aucagne and Leigh introduced a double-click method, in which two CuAAC reactions of compound **1** are spaced by a TMS-alkyne deprotection step to gain chemoselectivity.[6] In bisalkyne linker **2** by Girard et al., an electron-deficient propiolamide, which reacts with an azide thermally (i.e., without a copper catalyst), pairs up with a propargylic group targeted for a CuAAC reaction.[7] The pairing of cyclooctyne and terminal

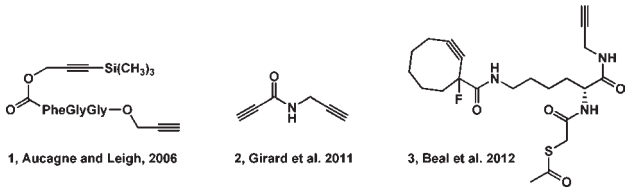


Figure 1. Unsymmetrical bisalkyne linkers.

(1) (a) Gramlich, P. M. E.; Warncke, S.; Gierlich, J.; Carell, T. *Angew. Chem., Int. Ed.* **2008**, *47*, 3442. (b) Sanders, B. C.; Friscourt, F.; Ledin, P. A.; Mbua, N. E.; Arumugam, S.; Guo, J.; Boltje, T.; Popik, V. V.; Boons, G.-J. *J. Am. Chem. Soc.* **2011**, *133*, 949. (c) Yi, L.; Sun, H.; Itzen, A.; Triola, G.; Waldmann, H.; Goody, R. S.; Wu, Y.-W. *Angew. Chem., Int. Ed.* **2011**, *50*, 8287.

(2) (a) Durmaz, H.; Dag, A.; Altintas, O.; Erdogan, T.; Hizal, G.; Tunca, U. *Macromolecules* **2007**, *40*, 191. (b) Galibert, M.; Dumy, P.; Boturyn, D. *Angew. Chem., Int. Ed.* **2009**, *48*, 2576. (c) Kempe, K.; Hoogenboom, R.; Jaeger, M.; Schubert, U. S. *Macromolecules* **2011**, *44*, 6424. (d) Ehlers, I.; Maity, P.; Aubé, J.; König, B. *Eur. J. Org. Chem.* **2011**, 2474. (e) Valverde, I. E.; Lecaille, F.; Lalmanach, G.; Aucagne, V.; Delmas, A. F. *Angew. Chem., Int. Ed.* **2012**, *51*, 718.

(3) Hein, J. E.; Fokin, V. V. *Chem. Soc. Rev.* **2010**, *39*, 1302.

(4) Sletten, E. M.; Bertozzi, C. R. *Acc. Chem. Res.* **2011**, *44*, 666.

(5) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004.

(6) Aucagne, V.; Leigh, D. A. *Org. Lett.* **2006**, *8*, 4505.

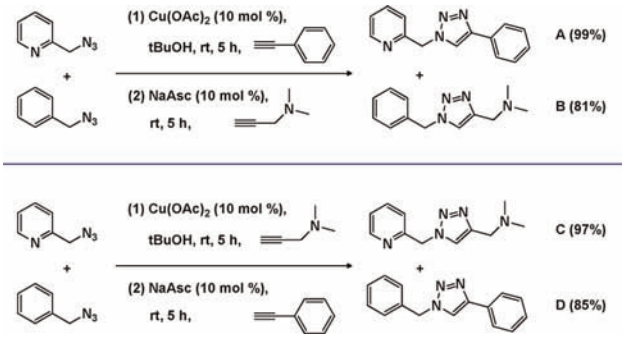
(7) Elamari, H.; Meganem, F.; Herscovici, J.; Girard, C. *Tetrahedron Lett.* **2011**, *52*, 658.

10.1021/ol300899n
Published on Web 05/04/2012

alkyne is utilized in compound **3** by Beal et al., in which the two triple bonds react via strain-promoted thermal reaction and copper(I)-catalyzed means, respectively.[8]

Although effective to various degrees, these earlier approaches suffer from a few limitations. The double-click method requires a protection/deprotection sequence, which adds to the workload. The thermal AAC reactions using strained or electron-deficient alkynes are relatively slow at rt, in addition to the lack of regioselectivity in affording 1,4- or 1,5-disubstituted triazoles. Furthermore, the propiolamide derivatives are prone to Michael addition with a nucleophile, thus limiting the scope of substrates in sequential ligations. In another noteworthy double-click method, amino-substituted organic azides are employed in which a diazo transfer reaction is required to activate the amino group to azido for the second CuAAC reaction.[9] Herein, we report a double-conjugation method in which two CuAAC reactions occur sequentially in a single reaction mixture without an intervening deprotection step. This method affords excellent regioselectivity while preserving the fast kinetics and large substrate scope of the CuAAC reaction.

Scheme 1. Azide Selectivity in CuAAC Reactions



Our group discovered that copper(II) acetate ($Cu(OAc)_2$) and chelating azides possess uniquely high reactivities in CuAAC reactions.[10] The reactivity difference between chelating and nonchelating azides is demonstrated in the two CuAAC reactions starting with an equal molar mixture of 2-picolylazide and benzylazide (Scheme 1). In the presence of only $Cu(OAc)_2$, the added alkyne selectively reacts with the chelating 2-picolylazide to afford triazoles **A** and **C**. The subsequent addition of sodium ascorbate increases the concentration of the copper(I) catalyst, leading to the second triazole formation (**B** and **D**) involving the nonchelating benzylazide. The chemoselectivity in organic azides (chelating vs nonchelating) opens up an opportunity to introduce two azido groups with inherently different reactivities into one substrate, such as **4**–**7** (Figure 2). These bisazides allow for sequential CuAAC reactions with two distinct alkynes in a one-pot procedure, without a protection/deprotection cycle. Moreover, it was shown previously that different alkynes have various reactivities to chelating azides,[10b] which makes it possible to obtain a single product by simply mixing two alkynes and a bisazide together in one experimental sequence.

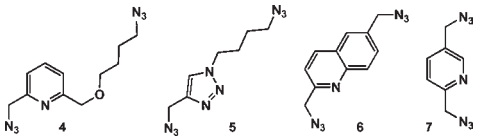


Figure 2. Unsymmetrical bisazides.

The syntheses of unsymmetrical bisazides **4**–**7** containing chelating and nonchelating azido groups are included in the Supporting Information. Bisazides **4** and **7** contain a 2-(azidomethyl)pyridyl chelating component, and an aliphatic and a benzylic nonchelating azide, respectively. In bisazide **5**, the N3 nitrogen atom on the triazolyl ring and the C4-azidomethyl group constitute a chelating component, while a nonchelating aliphatic azido group is attached via a 4-carbon linker. Compound **6** has a quinoline core with a chelating 2-azidomethyl and a nonchelating 6-azidomethyl group. In **4**–**7**, the chelating azido group would react with an alkyne molecule first under the Cu-$(OAc)_2$-accelerated conditions,[10b] leaving the nonchelating azido group for the CuAAC reaction with the second alkyne under more strongly reducing conditions.

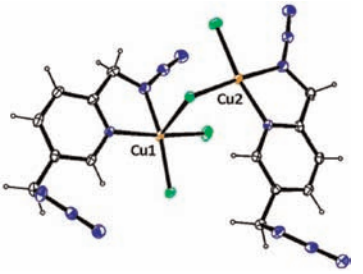


Figure 3. ORTEP diagram of $[Cu_2(\mathbf{7})_2Cl_4]$ (30% ellipsoids). Black, carbon and hydrogen; blue, nitrogen; green, chlorine; orange, copper.

The single crystal structure of complex $[Cu_2(\mathbf{7})_2Cl_4]$ (Figure 3) reveals the selective azido-copper interaction in bisazide **7**, which is the source of its chemoselectivity in CuAAC reactions. The copper(II) center is square pyramidal, where the bidentate chelating 2-(azidomethyl)-pyridyl moiety and two chloride ions constitute the square plane. The nonchelating azido group is left unbound.

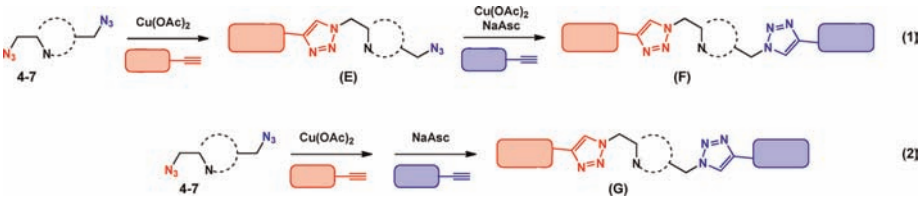
1-Ethynyl-4-nitrobenzene and propargyl alcohol were employed as alkyne substrates in demonstrating the

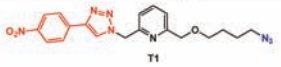
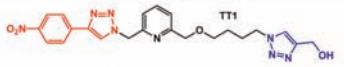
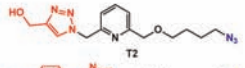
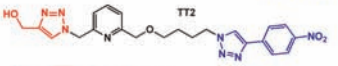
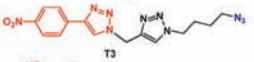
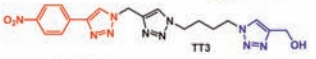
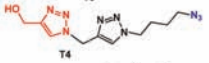
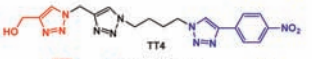
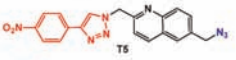
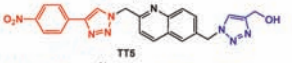
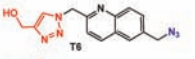
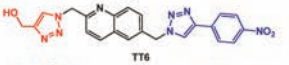
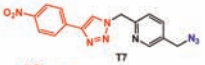
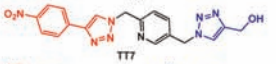
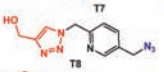
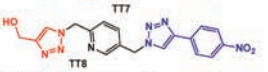
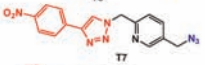
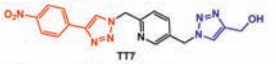
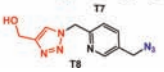
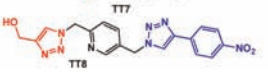
(8) Beal, D. M.; Albrow, V. E.; Burslem, G.; Hitchen, L.; Fernandes, C.; Lapthorn, C.; Roberts, L. R.; Selby, M. D.; Jones, L. H. *Org. Biomol. Chem.* **2012**, *10*, 548.

(9) Guiard, J.; Fiege, B.; Kitov, P. I.; Peters, T.; Bundle, D. R. *Chem.—Eur. J.* **2011**, *17*, 7438.

(10) (a) Brotherton, W. S.; Michaels, H. A.; Simmons, J. T.; Clark, R. J.; Dalal, N. S.; Zhu, L. *Org. Lett.* **2009**, *11*, 4954. (b) Kuang, G.-C.; Guha, P. M.; Brotherton, W. S.; Simmons, J. T.; Stankee, L. A.; Nguyen, B. T.; Clark, R. J.; Zhu, L. *J. Am. Chem. Soc.* **2011**, *133*, 13984.

Table 1. Chemoselective Formation of Mono- and Bistriazoles[a]



entry	azide	monotriazole (**E**)	yield of **E**[b]	bistriazole (**F** or **G**)	yield of **F**[b]	yield of **G**[b]
1	**4**		93%		87%	97%
2	**4**		84%		91%	95%
3	**5**		82%		74%	78%
4	**5**		82%		69%	74%
5	**6**		90%		81%	87%
6	**6**		88%[c]		77%[c]	84%[c]
7	**7**		99%		80%	92%
8	**7**		87%		76%	88%
9[d]	**7**		90%		75%	85%
10[d]	**7**		89%		73%	82%

[a] Azide (0.1 mmol) and alkyne (0.1 mmol) with 10 mol % of $Cu(OAc)_2$ at rt; entries 1–8 in CH_3OH/CH_2Cl_2 (1:1 v/v, 0.5 mL). [b] Isolated yield after purification by column chromatography. [c] 2–3% impurity is contained in the isolated product, presumably the reverse regioisomer and/or homobistriazole(s). [d] HEPES buffer (0.5 mL, 0.5 M, pH 7.0).

chemoselective double-click reaction involving bisazides **4**–**7** (eq 1, Table 1). The addition of the first alkyne to a solution of a bisazide in a 1:1 molar ratio in the presence of 10 mol % $Cu(OAc)_2$ resulted in the formation of monotriazoles **T1**–**T8** (Table 1). The high isolated yields (entries 1–8) indicated that the structures of bisazides and alkynes have only a marginal influence on the efficiency and chemoselectivity of the first CuAAC reaction.

Addition of the second alkyne with a catalytic amount of $Cu(OAc)_2$ (10 mol %) and sodium ascorbate (20 mol %) to the CH_2Cl_2/CH_3OH solution of the purified monotriazole products resulted in the formation of bistriazoles **TT1**–**TT8** in good yields (yield of **F**). The results shown in Table 1 from the two-step sequence demonstrate the high reactivity of the chelating azide, which undergoes the $Cu(OAc)_2$-mediated cycloaddition with an alkyne without affecting the nonchelating azide. The addition of sodium ascorbate affords a high concentration of copper(I), which is apparently needed for the nonchelating azido group to ligate with the second alkyne. Thus, by using appropriate reagents, sequential chemoseletive ligation of unsymmetrical bisazides **4**–**7**, each of which contains a chelating and nonchelating azido group, is achieved.

In addition to the stepwise syntheses of mono- and bistriazoles, we carried out a "one-pot" double-click ligation experiment without the isolation of the monotriazole intermediate (eq 2, Table 1). The introduction of the first alkyne into the CH_2Cl_2/CH_3OH solution of a bisazide in the presence of 10 mol % $Cu(OAc)_2$ was followed 5 h later by the addition of the second alkyne, which was accompanied by a sodium ascorbate solution to reduce $Cu(OAc)_2$. The CuAAC reaction between the nonchelating azide and the second alkyne proceeded to afford single detectable bistriazole products in excellent yields (Table 1, yield of **G**). It is notable that the order of alkyne addition, not the identity of the alkyne, directs the double-click reaction to reach different bistriazole products. From comparison of the overall isolated yields of bistriazoles from the stepwise procedure (Table 1, yield of **F**), the higher yields of the one-pot procedure (yield of **G**) suggest that not only the chemoselectivity of the double-click reaction is maintained but also efficient isolation of products with minimal material loss is managed without separating the monotriazole intermediate.

One important benefit of the CuAAC reaction for applications in chemical biology and material science is

the undiminished reactivity under physiological conditions. Bisazide **7** was chosen to test the viability of chemoselective double ligation in an aqueous solution buffered by HEPES at pH 7. The bistriazole products from both stepwise (Table 1, entries 9 and 10) and one-pot procedures were obtained in good isolated yields, which lends promise in bioconjugation applications.[11]

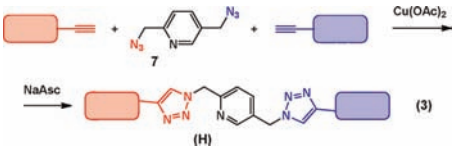
In our previous study, it was revealed that reactivities of various alkynes differ under $Cu(OAc)_2$-mediated conditions.[10b] For example, enhanced acidity of an alkyne leads to the increased rate of the CuAAC reaction, because alkyne deprotonation was shown to be kinetically significant.[10b] The differences in reactivities of both alkynes and azides make it possible to perform chemoselective three-component (two alkynes with different reactivities and a bisazide) reactions.

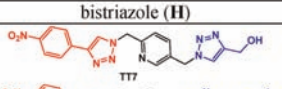
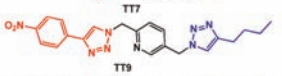
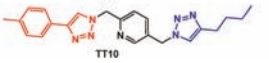
In the three-component reaction involving two different alkynes and bisazide **7** (eq 3, Table 2), the alkyne with a higher reactivity (e.g., 1-ethynyl-4-nitrobenzene and 4-ethynyltoluene) shall undergo $Cu(OAc)_2$-mediated CuAAC reaction selectively with the chelating azido group of **7**. After the first reaction is completed, the addition of sodium ascorbate to the reaction mixture would lead to the second CuAAC reaction between the nonchelating azido group and the less reactive alkyne (e.g., propargyl alcohol and 1-hexyne).

In agreement with the above expectation, the inclusion of two alkynes (1-ethynyl-4-nitrobenzene and propargyl alcohol) with bisazide **7** in CH_3CN resulted in the first cycloaddition selectively between the chelating azido group and 1-ethynyl-4-nitrobenzene, and the second CuAAC reaction between the nonchelating azido and propargyl alcohol to afford the bistriazole in 70% isolated yield (Table 2, entry 1). Compared to propargyl alcohol, 1-hexyne has a lower reactivity in CuAAC reaction. The large disparity between the reactivities of 1-ethynyl-4-nitrobenzene and 1-hexyne manifested in the one-pot synthesis of bistriazole **TT9**, which was obtained in an almost quantitative yield (Table 2, entry 2). The one-pot reaction of entry 2 was monitored via ^1H NMR spectroscopy, from which the exclusive ligation between the chelating azido group and 1-ethynyl-4-nitrobenzene was observed in CD_3CN in the presence of the idle 1-hexyne (Figures S1–S2).[12]

4-Ethynyltoluene and 1-hexyne are also effective in the one-pot double-click reaction involving bisazide **7**, in which the aromatic 4-ethynyltoluene and the aliphatic 1-hexyne were consumed sequentially to afford bistriazole **TT10**. The methyl groups of the two alkynes could conceivably be replaced by other structural entities, thus offering an attractive tool for the selective conjugation of multiple components under mild conditions.

Table 2. One-Pot, Three-Component Reactions[a]



entry	bistriazole (**H**)	yield of **H**[b]
1	 TT7	70%
2	 TT9	98%
3	 TT10	74%[c]

[a] Bisazide **7** (0.1 mmol) and two alkynes (0.1 mmol) in CH_3CN (0.5 mL) with addition of $Cu(OAc)_2$ (10 mol %) followed by adding NaAsc (20 mol %) after 5 h at rt. [b] Isolated yield after purification by column chromatography. [c] 3% impurity is contained in the isolated product presumably the reverse regioisomer and/or homobistriazole(s).

In conclusion, a method for the one-pot chemoselective double-click reaction of three components is described. This strategy employs unsymmetrical bisazides containing chelating and nonchelating azido groups, which exhibit different reactivities in CuAAC reactions. The easy preparation and simple structures of bisazides **4**–**7** suggest that they can be used as convenient conjugation linkers to join two ethynyl-functionalized building blocks with high regioselectivity under mild, including physiological conditions. Exploring the utilities of the bisazide linkers in areas of bioconjugation and material sciences is an ongoing effort in our laboratory.

Acknowledgment. This work was supported by the U.S. National Science Foundation (CHE-0809201).

Supporting Information Available. Experimental procedures, characterization of new compounds, .cif file of $[Cu_2(7)_2Cl_4]$, and additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

(11) Kalia, J.; Raines, R. T. *Curr. Org. Chem.* **2010**, *14*, 138.

(12) The second CuAAC reaction after the addition of sodium ascorbate did not take place at the prescribed concentration levels in the NMR tube, even at an elevated temperature.