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1,3-Dipolar cycloaddition of azides with electron-deficient alkynes under mild condition in water

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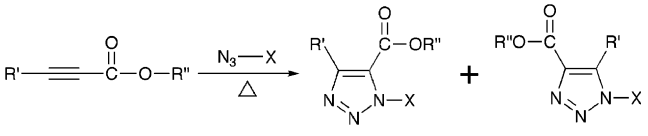
Abstract—We report a simple synthetic protocol for the 1,3-dipolar cycloaddition of azides with electron-deficient alkynes. Alkyne with at least one neighboring electron-withdrawing group proceeds with the cycloaddition successfully without any catalysts at room temperature in water. Under this simple condition, we evaluated a series of small molecule model reactions and then coupled an azido-DNA molecule with electron-deficient alkynes for the formation of [1,2,3]-triazole heterocycle, providing a potential method for introducing functional groups to DNA under biological conditions.

Since the 1,3-dipolar cycloaddition of azides with alkynes was investigated by Huisgen et al.[1] it has attracted much attention because of theoretical interest of the reaction[2] and the synthetic importance of the aromatic and nonaromatic five-membered [1,2,3]-triazole heterocycles. [1,2,3]-Triazole derivatives have been reported to exhibit antimicrobial activity,[3] as inhibitors of human leukocyte elastase,[4] as synthons for the preparation of antitumor dehydropyrrolizidine alkaloids,[5] and for the modification of nucleosides as antiviral agents.[6]

The traditional method for producing the triazole by cycloaddition requires elevated temperature, typically in refluxing conditions. It is known that alkynes with an electron-withdrawing functional group favor this irreversible Huisgen cycloaddition of azides and alkynes (Scheme 1).[7] We previously explored this reaction for site-specific fluorescent labeling of oligonucleotide for DNA sequencing.[8] Recently, new synthetic methods based on catalysts have been reported for the formation of [1,2,3]-triazoles. Cucurbituril and acetylcholinesterase were employed to lower the activation barrier for the azide–alkyne cycloaddition by sequestering the two components inside a host structure.[9,10] The copper(I)-catalyzed reaction unites azides and terminal alkynes regiospecifically to give only one-specific regioisomer, the 1,4-disubstituted [1,2,3]-triazole.[11] This copper(I)-catalyzed system was subsequently applied for the attachment of synthetic oligosaccharides to microtiter plate for biological assayes.[12] The same reaction was also recently explored for protein and cell surface labeling.[13] So far, the 1,3-dipolar cycloaddition between the alkynes and azides was conducted either at high temperature thermodynamically or at room temperature catalytically.



Scheme 1.

In the process of optimizing the 1,3-dipolar cycloaddition for DNA analysis and immobilization, we have found that if an electron-deficient internal or terminal alkyne is used, the 1,3-dipolar cycloaddition reaction can be carried out successfully using a simple protocol without any catalysts at room temperature in water, which is fully compatible with DNA modification inside a cell under biological conditions. However, under the same condition, alkynes without a neighboring electron-withdrawing group do not produce any cycloaddition products.

In the first series of experiments, we evaluated the reaction between ethyl 5-azidovalerate and electron-deficient alkynes (Table 1).

Keywords: 1,3-Dipolar cycloaddition; Electron-deficient alkynes; 5′-Azido DNA.
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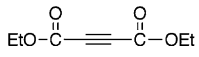
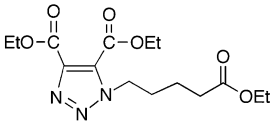
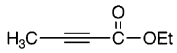
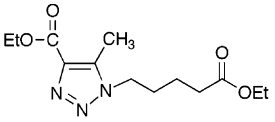
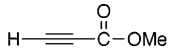
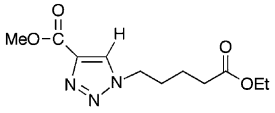
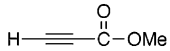
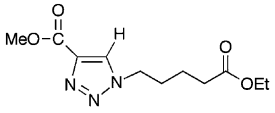
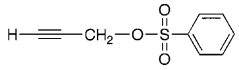
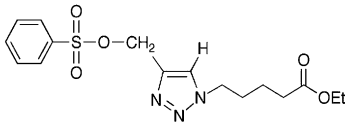
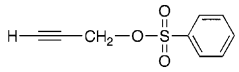
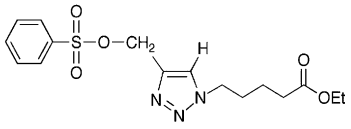
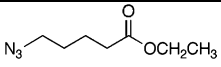
Table 1. Reaction of ethyl 5-azidovalerate with electron-deficient alkynes

Entry[a]	Alkyne	Product	Yield (%)
1			81
2			94
3			82
4[b]			90
5			67
6[b]			85

[a] For all entries, the azido compound is ethyl 5-azidovalerate,  rt, water solvent, t_R: 6–12 h.
[b] CuCl (0.1 equiv), t_R: 1 h.

Ethyl 5-azidovalerate was readily prepared from ethyl 5-bromovalerate by a nucleophilic substitution with NaN_3 in DMSO.[14] The pure products of cycloaddition were obtained simply by stirring the two reagents in water at room temperature for 6–12 h and then isolated by extraction followed by chromatography, or by directly filtering the precipitate followed by recrystallization from ethanol.[15] We determined the regiochemistry of the entry 3 product by X-ray crystallography (Fig. 1), indicating that only 1,4-regioisomer was produced selectively, which implies that the cycloaddition intermediate was mainly controlled by the steric hindrance effect. To compare the result of the entry 3 product with that of the catalyst-mediated reaction, the same product was produced in the presence of CuCl (entry 4). When Cu(I) catalyst was used for cycloaddition with terminal alkynes, the reaction was completed in 1 h with a yield of 90%, producing a single 1,4-regioisomer identical to the product in entry 3. Similarly, the entry 6 product (85%) was produced with a higher yield in the presence of Cu(I) catalyst than that of the entry 5 product (67%). However, the Cu(I) catalyzed reaction only works for the terminal alkynes.[11]

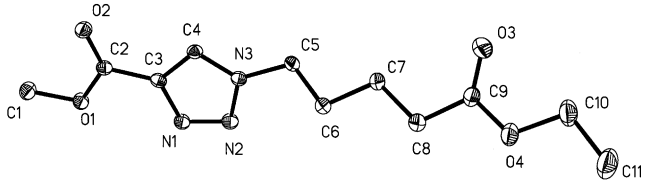
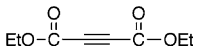
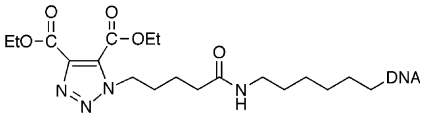
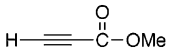
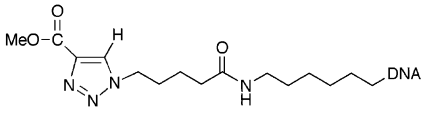
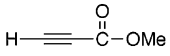
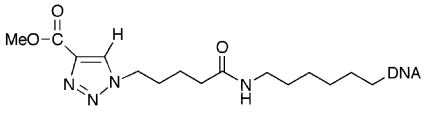
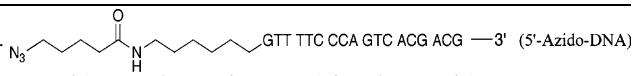


Figure 1. X-ray structure of the entry 3 product (1-ethoxycarbonyl butyl-4-methoxycarbonyl-1,2,3-triazole): orthorhombic; $a \times b \times c$, 5.6001 Å × 6.9381 Å × 33.880 Å; $\alpha, \beta, \gamma = 90°, 90°, 90°$; $R = 0.0762$ and $R_w = 0.1539$.

Encouraged by these results, we applied this synthetic protocol to the cycloaddition of an azido-DNA with electron-deficient alkynes (Table 2). The azido-labeled DNA was prepared by reacting succinimidyl 5-azidovalerate with an amino-linker modified oligonucleotide (5′-amino-GTT TTC CCA GTC ACG ACG-3′; M13 −40 universal forward sequencing primer, m/z 5631) with a 96% yield. The azido-labeled DNA was characterized by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI–TOF MS), which showed a single major peak at m/z 5757 Da (calcd value: m/z 5756 Da).[8] In a typical reaction, the azido-DNA (4 nmol) was reacted with 300-fold excess of alkynyl compounds in 60 μL water at room temperature for 48 h. After the reaction, excess alkynes were removed by size-exclusion chromatography and the resulting product was desalted with an oligonucleotide purification cartridge. The product was purified by HPLC and analyzed with MALDI–TOF MS.[16] The major peaks in the mass spectra generated by entries 7 and 8 products matched exactly with the calculated values as shown in Figure 2. The product of entry 9 was synthesized using the Cu(I) catalyst in 24 h with a 60% yield.

Table 2. Reaction of an azido-DNA with electron-deficient alkynes

Entry[a]	Alkyne	Product	Yield (%)
7			45
8			67
9[b]			60

[a] For all entries, the azido compound is 5′-azido-DNA, rt, 48 h, water solvent. 

[b] Methyl propiolate (300 equiv), CuI (300 equiv), *N*,*N*-diisopropylethylamine (300 equiv), rt, 24 h, H_2O/CH_3CN (4/1 volume ratio).

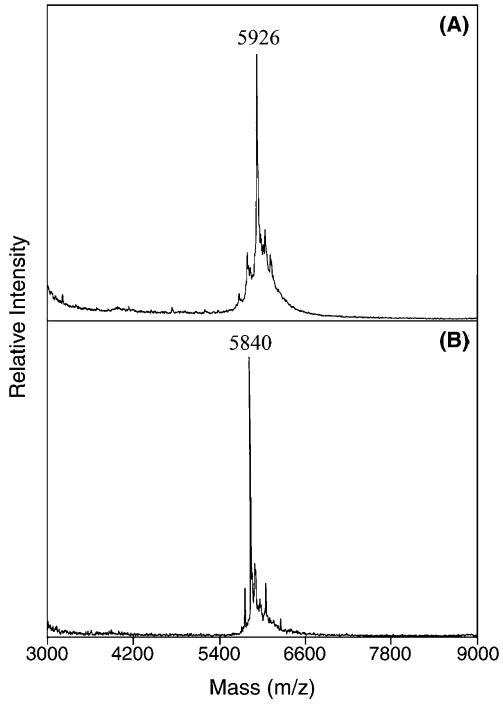


Figure 2. (A) MALDI–TOF MS spectra of the entry 7 product (*m*/*z*: found, 5926; calcd, 5926) and (B) the entry 8 product (*m*/*z*: found, 5840; calcd, 5840).

In summary, we reported here a simple and mild protocol for the 1,3-dipolar cycloaddition between azides and alkynes. When the alkyne (either terminal or internal) has at least one neighboring electron-withdrawing functional group, the triazole formation can be achieved at room temperature in water without any catalysts. In the case of the terminal alkynes, the cycloaddition was proceeded much faster in the presence of Cu(I) catalyst.

Acknowledgements

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References and notes

1. (a) Huisgen, R.; Szeimies, G.; Moebius, L. *Chem. Ber.* **1967**, *100*, 2494–2507; (b) Huisgen, R. *1,3-Dipolar Cycloaddition Chemistry*; New York: Wiley, 1984; (c) Huisgen, R. *Pure Appl. Chem.* **1989**, *61*, 613–628.
2. Lwowski, W. In *1,3-Dipolar Cycloaddition Chemistry*; Wiley: New York, 1984; Vol. 1.
3. Hartzel, L. W.; Benson, F. R. *J. Am. Chem. Soc.* **1954**, *76*, 667–670.
4. Hlasta, D. J.; Ackerman, J. H. *J. Org. Chem.* **1994**, *59*, 6184–6189.
5. Pearson, W. H.; Bergmeier, S. C.; Chytra, J. A. *Synthesis* **1990**, 156–159.
6. Noriis, P.; Horton, D.; Levine, B. R. *Heterocycles* **1996**, *43*, 2643–2656.
7. (a) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew Chem., Int. Ed.* **2001**, *40*, 2005–2021; (b) Palacios, F.; Retana, A. M.; Pagalday, J. *Heterocycles* **1994**, *38*, 95–102.
8. Seo, T. S.; Li, Z.; Ruparel, H.; Ju, J. *J. Org. Chem.* **2003**, *68*, 609–612.
9. (a) Mock, W. L.; Irra, T. A.; Wepsiec, J. P.; Manimaran, T. L. *J. Org. Chem.* **1983**, *48*, 3619–3620; (b) Mock, W. L.; Irra, T. A.; Wepsiec, J. P.; Adhya, M. *J. Org. Chem.* **1989**, *54*, 5302–5308; (c) Krasia, T. C.; Steinke, J. H. G. *Chem. Commun.* **2002**, 22–23; (d) Tuncel, D.; Steinke, J. H. G. *Chem. Commun.* **2002**, 496–497; (e) Tuncel, D.; Steinke, J. H. G. *Chem. Commun.* **2001**, 253–254.
10. Lewis, W. G.; Green, L. G.; Grynszpan, F.; Radic, Z.; Carlier, P. R.; Taylor, P.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 1053–1057.
11. (a) Rostovtsev, V. V.; Green, J. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596–2599; (b) Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057–3064.
12. Fazio, F.; Bryan, M. C.; Blixt, O.; Paulson, J. C.; Wong, C. *J. Am. Chem. Soc.* **2002**, *124*, 14397–14402.
13. (a) Speers, A. E.; Adam, G. C.; Cravatt, B. F. *J. Am. Chem. Soc.* **2003**, *125*, 4686–4687; (b) Link, A. J.; Tirrell, D. A. *J. Am. Chem. Soc.* **2003**, *125*, 11164–11165.

14. Khoukhi, N.; Vaultier, M.; Carrie, R. *Tetrahedron* **1987**, *43*, 1811–1822.
15. *General procedure*: In a pear shaped flask (25 mL) equipped with a magnetic stirring bar, 1.2 mmol of the alkynyl compound was suspended in H_2O (5 mL), and then mixed with ethyl 5-azidovalerate (0.19 g, 1.2 mmol) vigorously at room temperature for 6–12 h. After the reaction, the organic layer (entries 1, 2, and 5) was separated by extraction with CH_2Cl_2. The organic phase was washed with H_2O, dried over Na_2SO_4, and concentrated. The residue was purified by silica gel flash column chromatography using chloroform as an elute to obtain the pure oily products. In case of entries 3 and 4, the product was precipitated as a white solid in water. The product was filtered and recrystallized from EtOH. In case of entries 4 and 6, the same procedure was followed as above except that 0.1 equiv of CuCl was added in the reaction mixture and the reaction time was 1 h. The product of entry **6** was purified by silica gel flash column chromatography to obtain an oily product. The isolated products were characterized by ^1H and ^{13}C NMR and HRMS. Spectral data of the entry 1 product: ^1H NMR (400 MHz, $CDCl_3$) δ 4.64 (t, 2H, $J = 9.5$ Hz), 4.52–4.41 (m, 4H), 4.19–4.11 (q, 2H, $J = 9.5$ Hz), 2.38 (t, 2H, $J = 9.7$ Hz), 2.08–1.95 (m, 2H), 1.76–1.65 (m, 2H), 1.44 (t, 6H, $J = 9.5$ Hz), 1.28 (t, 3H, $J = 9.5$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.7, 160.1, 158.4, 140.2, 129.7, 62.8, 61.7, 60.3, 49.9, 33.2, 29.4, 21.5, 14.1, 13.8. HRMS (FAB^+) calcd for $C_{15}H_{24}O_6N_3$, 342.1665 ($M+H^+$); found, 342.1654. Spectral data of the entry 2 product: ^1H NMR (400 MHz, $CDCl_3$) δ 4.20 (q, 2H, $J = 7.1$ Hz), 4.12 (q, 2H, $J = 7.1$ Hz), 3.28 (t, 2H, $J = 6.5$ Hz), 2.32 (t, 2H, $J = 7.0$ Hz), 1.97 (s, 3H), 1.72–1.60 (m, 4H), 1.28 (t, 3H, $J = 7.1$ Hz), 1.24 (t, 3H, $J = 7.1$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.8, 160.2, 158.5, 140.3, 129.8, 61.8, 50.0, 33.3, 33.2, 29.5, 21.8, 14.0. HRMS (FAB^+) calcd for $C_{13}H_{22}O_4N_3$, 284.1610 [$M+H^+$]; found, 284.1610. Spectral data of the entries 3 and 4 product: ^1H NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 1H), 4.53 (t, 2H, $J = 9.4$ Hz), 4.21 (q, 2H, $J = 9.5$ Hz), 4.04 (s, 3H), 2.45 (t, 2H, $J = 9.5$ Hz), 2.14–2.04 (m, 2H), 1.80–1.70 (m, 2H), 1.35 (t, 3H, $J = 9.5$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.7, 161.1, 139.9, 127.3, 60.5, 52.2, 50.2, 33.2, 29.4, 21.6, 14.1; HRMS (FAB^+) calcd for $C_{11}H_{18}O_4N_3$, 256.1297 [$M+H^+$]; found, 256.1310. Spectral data of the entries 5 and 6 product: ^1H NMR (400 MHz, $CDCl_3$) δ 7.96–7.93 (m, 2H), 7.68–7.65 (m, 1H), 7.58–7.54 (m, 1H), 4.74 (d, 2H, $J = 2.4$ Hz), 4.17–4.10 (q, 2H, $J = 7.2$ Hz), 3.30 (t, 2H, $J = 6.5$ Hz), 2.48 (t, 1H, $J = 2.4$ Hz), 2.34 (t, 2H, $J = 6.5$ Hz), 1.73–1.64 (m, 4H, $J = 6.4$ Hz), 1.26 (t, 3H, $J = 6.4$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.5, 136.4, 134.5, 129.7, 128.5, 75.6, 60.7, 58.0, 51.4, 34.0, 28.6, 22.5, 14.5; HRMS (FAB^+) calcd for $C_{16}H_{22}O_5N_3S$, 368.1300 [$M+H^+$]; found, 368.1280. The crystallographic data of the entry 3 product has been deposited at the Cambridge Crystallographic Data Center with the deposition number CCDC 220803.
16. *HPLC analysis*: HPLC analysis was carried out on a Waters system consisting of a Rheodyne 7725I injector, a 600 Controller, Xterra MS C18 (4.6 × 50-mm) column, and 996 photodiode array detector. Elution was performed by using a linear gradient (8–28%) of methanol in a buffer that consists of 8.6 mM aqueous triethylamine and 100 mM hexafluoroisopropyl alcohol (pH 8.1) at a flow rate of 0.5 mL/min with the temperature set at 50 °C. Under this condition, the elution time of the entry 7 product was 49.6 min and that of the entry 8 product was 28.2 min. *MALDI–TOF MS analysis*: Mass measurement of oligonucleotides was performed using a Voyager™ DE MALDI–TOF mass spectrometer. DNA product (20 pmol) was suspended in 2 μL of 3-hydroxypicolinic acid matrix solution. This mixture (0.5 μL) was spotted on a stainless steel sample plate, air-dried, and analyzed. The measurement was taken using a positive ion mode with 25 kV accelerating voltage, 94% grid voltage and a 350 ns delay time with internal calibration.