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A Fluorogenic 1,3-Dipolar Cycloaddition Reaction of 3-Azidocoumarins and Acetylenes†

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ABSTRACT

Copper(I)-catalyzed 1,3-dipolar cycloaddition reaction of nonfluorescent 3-azidocoumarins and terminal alkynes afforded intense fluorescent 1,2,3-triazole products. The mild condition of this reaction allowed us to construct a large library of pure fluorescent coumarin dyes. Since both azide and alkyne are quite inert to biological systems, this reaction has potential in bioconjugation and bioimaging applications.

Bioconjugation has recently emerged as a fast-growing technology that affects almost every discipline of life science. It aims at the ligation of two or more molecules (or supramolecules) to form a new complex with the combined properties of its individual components.[1] One important application of bioconjugation is to modify cellular components selectively with signaling probes for proteomics, functional genomics, and cell biology research.[2] A multistep procedure is commonly employed wherein the cellular entity is first attached with a detectable tag, such as fluorescent dyes or biotin, followed by purification of the ligated product and detection. However, excess prelabeled reagents are generally hard to remove from the intracellular environment or from tissues of living organisms, which prohibits the application of a multistep labeling procedure in many situations. An ideal alternative would be a chemoselective process that is orthogonal to biological components and the ligated product will afford strong detectable signal while the unbound reagent does not contribute any background. Very few such ligations have been reported.[3,4]

As a prototype of "click chemistry",[5] the recent advance of Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition of azides and alkynes affords superior regioselectivity and almost quantitative transformation under extremely mild conditions.[6] Alkyne and azide groups are very small in size, are highly energetic, and have a particularly narrow distribution of reactivity. They can be conveniently introduced to organic compounds and are quite indifferent to solvent and pH. Therefore, they have been employed as a pair of

† This paper is dedicated to Professor Manfred Schlosser on the occasion of his 70th birthday.

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orthogonal linkers for chemoselective ligations through a Cu(I)-mediated cycloaddition reaction by many research groups.[7] Here, we report a "clicking-and-probing" ligation protocol, a fluorogenic version of 1,3-dipolar cycloaddition of azides and alkynes, which has potential in covalently linking any two biomolecules or supramolecular complexes. The reaction design is schematically illustrated in Figure 1. The fluorescent

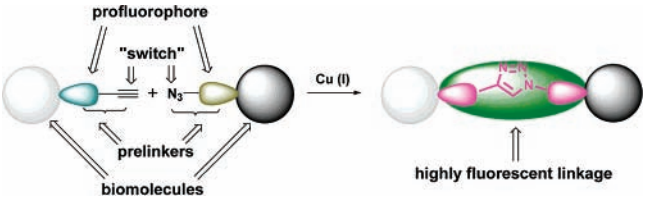
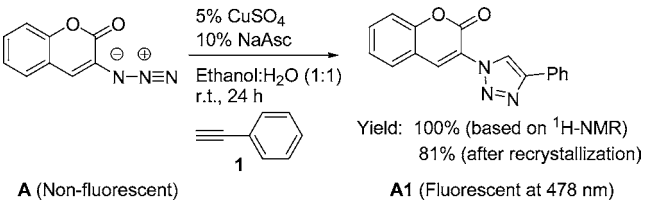


Figure 1. Schematic illustration of the "clicking-and-probing ligation". Profluorophores (or "prelinkers") containing azide or alkyne moieties are fluorescent inactive, which upon cycloaddition would lead to the formation of triazolyl units with enhanced fluorescent emissions.

signals will be triggered by the formation of triazole rings, which have the potential to be used as probes for imaging or as reporters to monitor the ligation efficiency.

Coumarin was chosen as the profluorophore since it is small in size, biocompatible, and easy to manipulate synthetically. Substitutions at the 3- and 7-positions of coumarin dyes are known to have a strong impact on their fluorescence properties.[8] Compound **A** was first synthesized with an azido group attached to the 3-position (Scheme 1). As we expected,

Scheme 1



A showed no fluorescence due to the quenching effect from the electron-rich α-nitrogen of the azido group. With catalytic Cu(I), the azide reacted smoothly with phenylacetylene (**1**) in a mixed solution of ethanol and water (1:1) to afford cycloaddition products **A1** at room temperature quantitatively. **A1** fluoresces at 478 nm due to the elimination of the quenching through the formation of the triazole ring. The quantum yield of **A1** is 0.30 in comparison to 0.0 of the starting materials **A**. This result encouraged us to further explore the impact of the structural features of azidocoumarins and alkynes to the fluorescent properties of the products.

As shown in Figure 2, eight 3-azidocoumarins and one 4-azidocoumarin were synthesized in addition to twenty-four

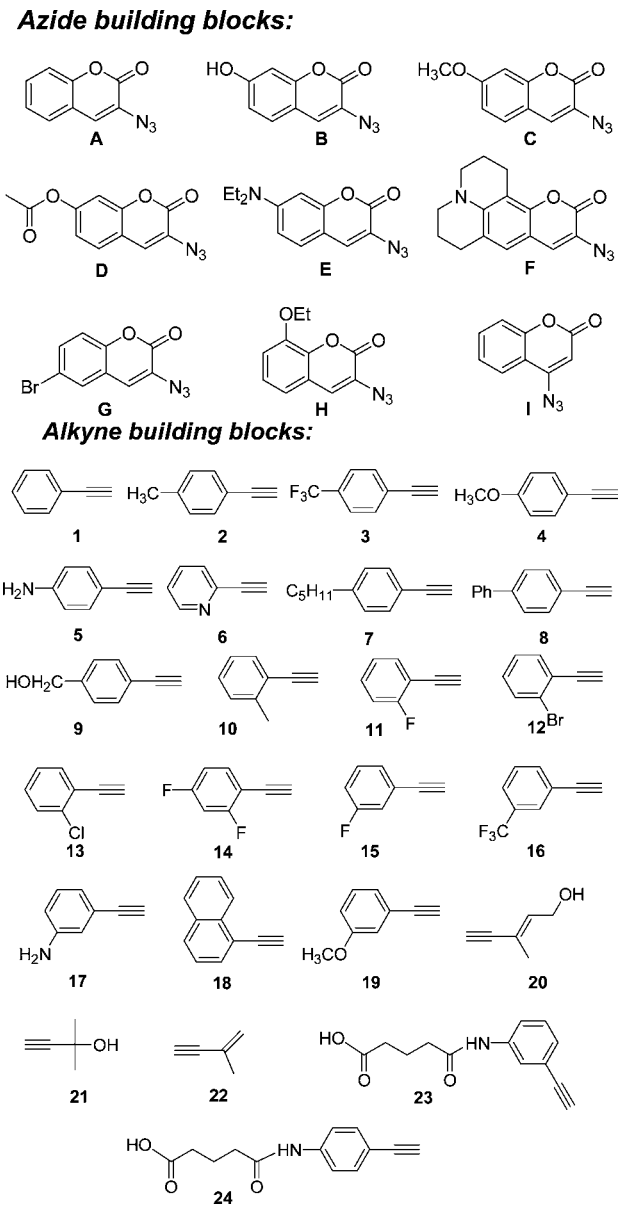


Figure 2. Building blocks for triazolylcoumarin dyes.

alkynes (**1**−**24**) which were purchased or synthesized for our study. The synthesis of 3-azidocoumarins was quite straightforward starting from the respecting substituted salicylaldehyde and *N*-acetylglycine or ethyl nitroacetate

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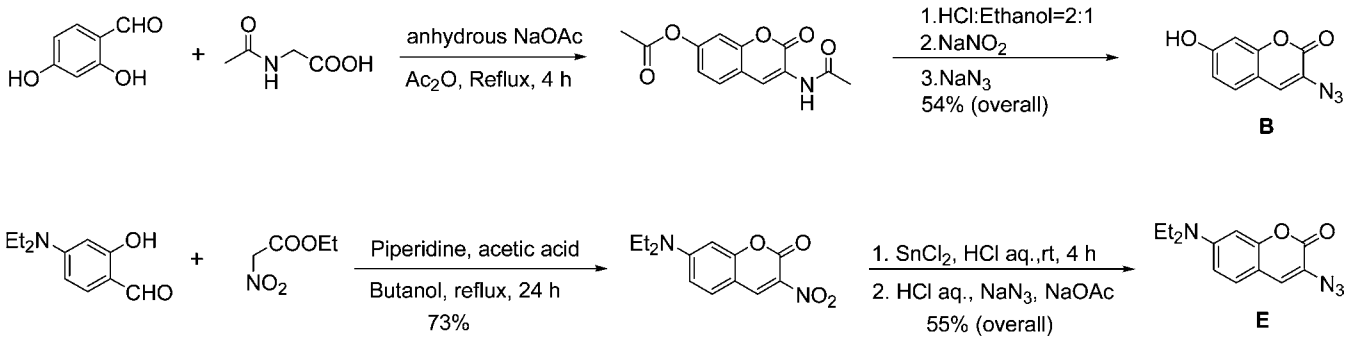
Scheme 2. Typical Synthetic Procedures of 3-Azidocoumarins



(Scheme 2). All of the azidocoumarin compounds (**A-I**) show no or very weak fluorescence.

The mild reaction conditions and high fidelity of the Cu(I)-catalyzed process allowed us to screen the fluorogenic properties of the cycloaddition reactions combinatorially. The synthesis of the coumarins and the screening procedures are described in detail in the Supporting Information. Briefly, a 96-well microtiter plate was used in which each well-represented a unique combination of azidocoumarin and alkyne. The total volume for each reaction was 200 μL

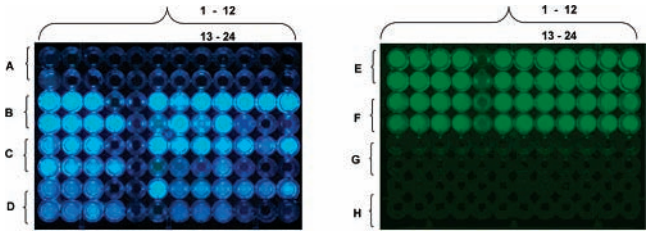


Figure 3. Combinatorial synthesis and screening of a triazolyl-coumarin library in microtiter plates. Azides **A**−**H** and alkynes **1**−**24** were used in the reactions. To take the pictures, UV filters have been used. Therefore, the colors shown here do not represent the true fluorescent wavelengths.

(DMSO/H_2O = 1:1) containing 1 mM azide and alkyne catalyzed with 16 mM $CuSO_4$ and sodium ascorbate. The reactions were generally completed in 12 h at room temperature monitored by TLC or mass spectroscopy. The formation of the fluorescent or nonfluorescent triazole compounds could be easily ascertained upon irradiation at 365 nm with a hand-held UV lamp (Figure 3). The reaction mixtures were further diluted to 1 or 10 μM for quantitative fluorescent detection. The absorbance and fluorescence properties of the products were fairly widespread (λ_{max}^{Ab} = 298−445 nm; λ_{max}^{Em} = 388−521 nm). The raw data are displayed in Figure 4. Electron-donating groups at the 7-position of 3-azidocoumarins (such as **B**, **E**, and **F)** showed strong enhancement of the fluorescence and dominated the emission color. The quantum yields of the corresponding triazolylcoumarin products are around 0.6−0.7 (data not shown). The structure−property relationships are still under investigation.

Based on the preliminary screening, selected coumarin products were then synthesized in large quantity. In a cosolvent of ethanol and water (1:1), the triazole dyes were formed quantitatively monitored with ^{1}H NMR. The products can be collect by simple filtration after evaporation of ethanol. In most situations, no further purifications were necessary and the yields were around 80% (Table 1). Due to the high reactivity of aromatic azides used in the synthesis, the cycloaddition went to completion even at 0 °C. It will benefit the real application of ligation between biomolecules, for which elevated temperature is usually destructive.

The inertness of azides and acetylenes allows further derivatization and attachment to other entities such as

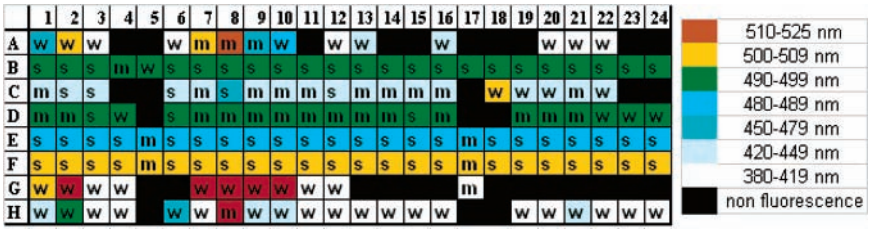
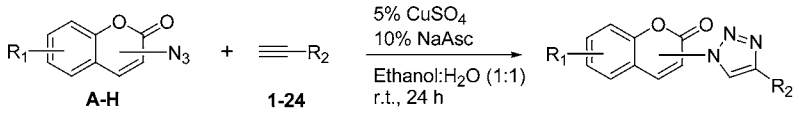
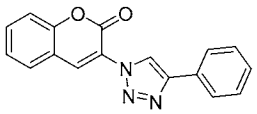
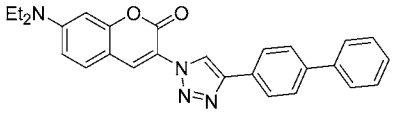
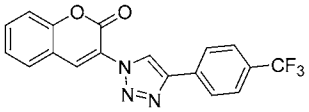
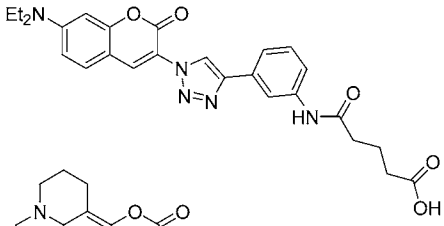
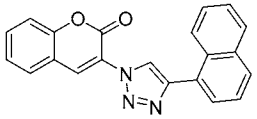
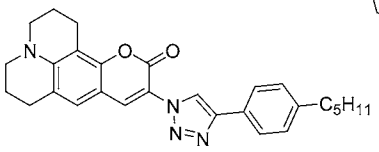
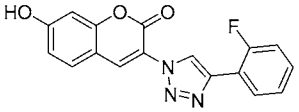
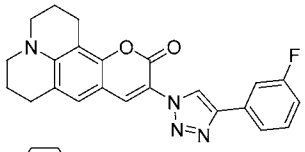
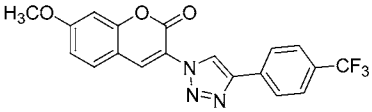
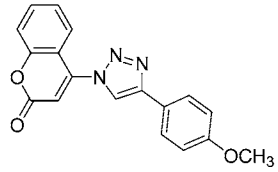
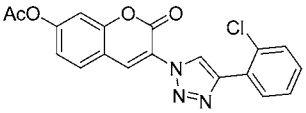


Figure 4. Intensities and emission wavelengths of the final triazolocoumarin products. The rows and columns refer to different building blocks as indicated in Figure 2. The relative fluorescent intensities are labeled with different letters, in which w = weak; m = medium; and s = strong. The color of each cell represents the emission wavelength. The detailed data are listed in Table S-1 of the Supporting Information.

Table 1. Synthesis of Triazolylcoumarins[a]



A-H 1-24

compound	product	yield	compound	product	yield
A1		81%	E8		80%
A3		63%	E23		77%
A18		70%	F11		62%
B11		64%	F15		63%
C3		86%	I4		84%
D13		80%			

[a] The yield is the isolated yield by direct filtration. For more data, see the Supporting Information.

biomolecules, polymers, nanoparticles, and surfaces. We have successfully used this "click and probing" ligation protocol in functionalizing bionanoparticles. The bioconjugation efficiencies can be reported by the fluorescent emission assays of the triazolylcoumarin products.[9] Therefore, this reaction should have broad application in the emerging field of cell biology and functional proteomics due to the high reaction efficiency at mild reaction conditions and the distinguished fluorescence properties of the products. In addition, this work is the first to report using azide−alkyne ligation to synthesize fluorescent compounds combinatorially;[10] the mild reaction conditions and the easy workup can be easily adopted to construct a large library of pure fluorescent coumarin dyes with a parallel synthesizer. Furthermore, the concept of our design will lead to the convenient synthesis of other fluorescent compounds.

Acknowledgment. We are grateful to the University of South Carolina, USC Research and Productive Scholarship, and ARO-MURI award for support of this work. We thank Miss Xin Wang for the assistance in the synthesis and fluorescent measurements.

Supporting Information Available: All experimental procedures and fluorescence data. This material is available free of charge via the Internet at http://pubs.acs.org/.

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(9) The results will be published elsewhere.

(10) During preparation of this manuscript, another fluorogenic probe was reported with Cu(I)-catalyzed azide−alkyne reaction: Zhou, Z.; Fahrni, C. J. *J. Am. Chem. Soc.* **2004**, *126*, 8862−8863. For recent combinatorial synthesis for fluorescent compounds, see ref 8 as well as: (a) Rosania, G. R.; Lee, J. W.; Ding, L.; Yoon, H.-S.; Chang, Y.-T. *J. Am. Chem. Soc.* **2003**, *125*, 1130−1131. (b) Lavastre, O.; Illitchev, I.; Jegou, G.; Dixneuf, P. H. *J. Am. Chem. Soc.* **2002**, *124*, 5278−5279.