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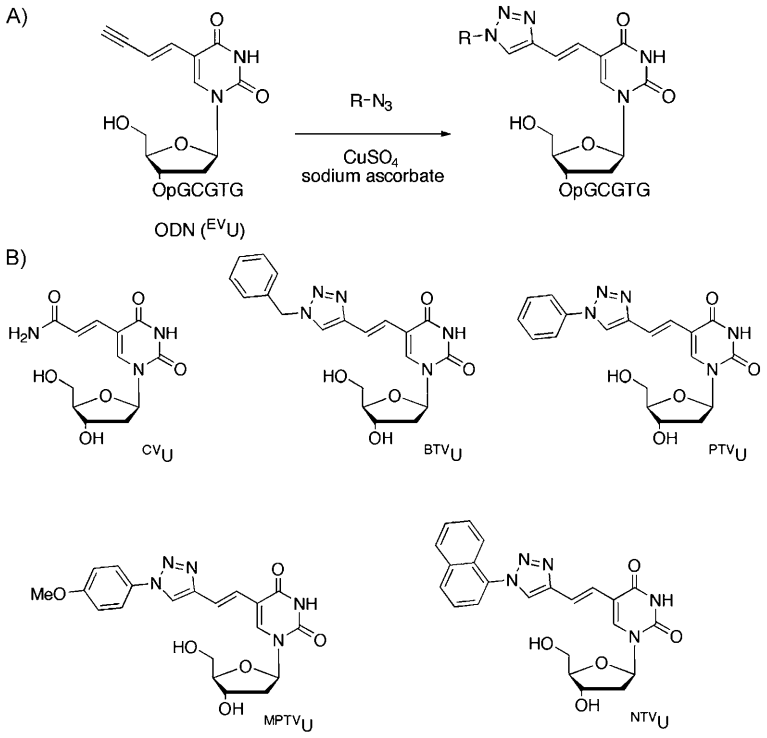
Click Chemistry as an Efficient Method for Preparing a Sensitive DNA Probe for Photochemical Ligation

Takehiro Ami and Kenzo Fujimoto*[a]

Genetic science entered a new stage with the progress of the genome project. As new genes are rapidly being identified, examination of their functions is becoming more important, especially in the field of biology, where the focus is shifting from individual genes and proteins to the interactions between the enormous numbers of proteins and their involvement in various life phenomena. Technology that cuts and ties DNA and DNA enzymes such as DNA ligase is essential to this research. Although enzymatic ligation methods have advantages, there are many limitations. For example, enzyme-linked assays need to include a careful selection of the most suitable conditions, including temperature, pH, and salt concentration, because of the use of enzymes and the fact that ligases have low activities for RNAs.[1] Furthermore, although very short probes have the highest sequence specificity, ligase enzymes are inefficient with short oligonucleotides.[2] For a alternative to enzymatic ligation methods, we have reported a highly efficient and reversible template-directed DNA photoligation procedure.[3] Template-directed photoligation with 5-carboxyvinyl-2′-deoxyuridine (^{CV}U)[3] can be used for detecting DNA or RNA sequences with high sensitivity. However, in photoligation with ^{CV}U, a long photoirradiation time at 366 nm is required to complete photoligation. To overcome the limitation of photoligation with ^{CV}U, we now report a new photosensitive probe that takes advantage of the electronic structural changes associated with the formation of triazole rings[4] in the Cu^{I}-catalyzed version of the azide–alkyne cycloaddition reaction[5] ("click" reaction[6]) discovered by the groups of Meldal[7] and Sharpless.[8] In the field of nucleic acid chemistry, Cu^{I}-catalyzed azide–alkyne cycloaddition has been used for labeling oligonucleotides with a range of moieties, such as fluorescein, for example,[9–19] for coupling oligonucleotides to monolayers,[20] and for templated strand ligation.[21] As an example, we report an efficient procedure for preparing DNA probes for quick SNP typing through photochemical ligation.

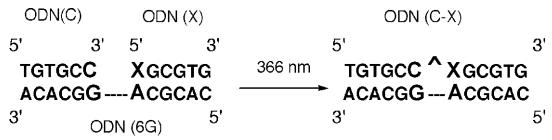
The nucleoside phosphoramidite of 5-ethynylvinyl-2′-deoxyuridine (^{EV}U) was prepared, and this monomer was incorporated into an ODN by standard automated DNA synthesis protocols. After deprotection and purification of the oligomer, an ODN containing ^{EV}U—5′-d(^{EV}UGCGTG)-3′, or ODN(^{EV}U)—was characterized by MALDI-TOF-MS.

Four azides were purchased or synthesized by a method reported in the literature,[22] and Huisgen cycloadditions between the ODN(^{EV}U) and the azides were carried out. After purification of the products, photoresponsive ODNs (Scheme 1) were characterized by MALDI-TOF-MS (see the Supporting Information).



Scheme 1. A) Synthesis of photoresponsive ODNs. B) Structures of photoresponsive nucleosides ^{CV}U, ^{BTV}U, ^{PTV}U, ^{MPTV}U, and ^{NTV}U.

We next determined the feasibility of photoligation of these triazole-ring-containing ODNs and ODN(C) in the presence of ODN(6G) (Scheme 2). ODN(^{NTV}U) and ODN(C) were irradiated



Scheme 2. Schematic illustration of photoligation of photoresponsive ODNs and ODN(C) in the presence of ODN(6G).

[a] *T. Ami, Prof. Dr. K. Fujimoto*
School of Materials Science
Japan Advanced Institute of Science and Technology
Asahidai, Nomi, Ishikawa 923-1292 (Japan)
Fax: (+81) 761-51-1671
E-mail: kenzo@jaist.ac.jp

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at 366 nm at 0 °C in the presence of template ODN (6G). HPLC analysis of a mixture of ODN (^{NTV}U) and ODN (C) photoirradiated with template ODN (6G) indicated clean and efficient formation of ligated ODN (C—^{NTV}U) and the concomitant disappearance of ODN (^{NTV}U) and ODN (C) (Figure 1). MALDI-TOF MS indicated

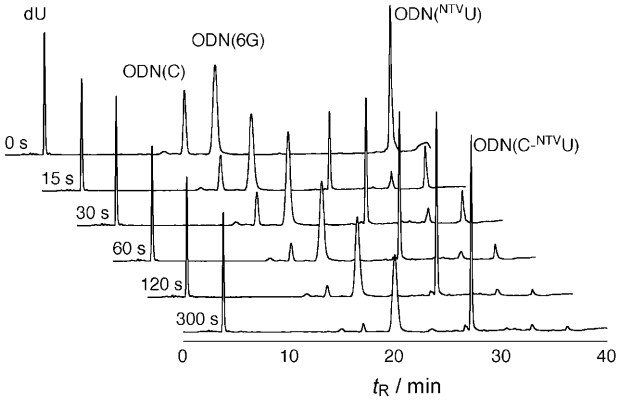

Figure 1. HPLC analysis of photoligation with ODN (^{NTV}U) and ODN (C) in the presence of ODN (6G).

that ODN (C—^{NTV}U) was a ligated product of ODN (^{NTV}U) and ODN (C). The molecular weight of (C—^{NTV}U) was equal to the sum of the molecular weights of ODN (^{NTV}U) and ODN (C) (see the Supporting Information). We also determined the feasibility of photoligation of the photoresponsive ODNs and ODN (C) in the presence of ODN (6G) (see the Supporting Information).

The times required to reach 50% conversion and the molar extinction coefficients (366 nm) for photoligation with photoresponsive ODNs and ODN (C) in the presence of ODN (6G) are shown in Table 1. The photoligation rates with ODN (^{BTV}U) are

Table 1. Times required to reach 50% conversion for photoligation of photoresponsive ODNs and ODN (C) in the presence of ODN (6G), together with molar extinction coefficients (366 nm) of photoresponsive nucleosides.

X	Time to reach 50% conversion [s]	ε at 366 nm [$M^{-1}cm^{-1}$]
^{CV}U	75	112
^{BTV}U	38	845
^{PTV}U	33	1008
^{MPTV}U	30	1095
^{NTV}U	16	1410

more rapid than those with ODN (^{CV}U). This result shows that the triazole rings, which have electron-donating characters, effectively accelerate the photoligation. The photoligation rates with ODN (^{PTV}U) are more rapid than those with ODN (^{BTV}U). This result is because the phenyl group has stronger electron-donating properties than the benzyl group. Moreover, the photoligation rates with ODN (^{MPTV}U) and ODN (^{NTV}U), which have more strongly electron-donating substituents, were more rapid. The time needed for photoligation to reach 50% conversion with ODN (^{NTV}U) was one-fourth of the time with ODN (^{CV}U). Photoligation on a timescale of seconds becomes possible with ^{NTV}U (Figure 2).

We also determined the feasibility of photoligation of the triazole-ring-containing ODNs and ODN (T) in the presence of

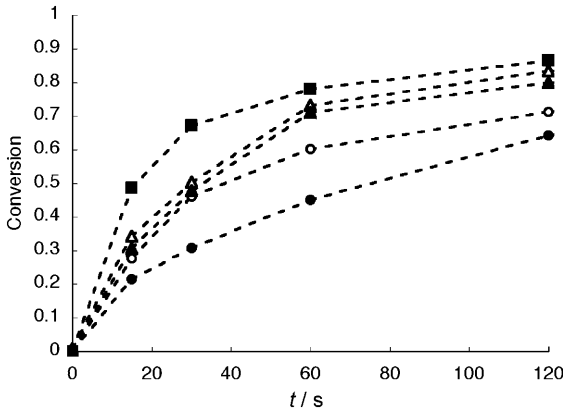

Figure 2. Comparison of photoligation rates with ODN (^{CV}U) (●) or ODN (^{BTV}U) (○), ODN (^{PTV}U) (▲) or ODN (^{MPTV}U) (△), or ODN (^{NTV}U) (■) and ODN (C) in the presence of ODN (6G).

ODN (6A). In this case, it was also shown that the photoligation rates with triazole-ring-containing ODNs are more rapid than the photoligation rates with ODN (^{CV}U). The times needed for photoligation to reach 50% conversion of the triazole-ring-containing ODNs were shorter than 20 s (see the Supporting Information).

To show that this new photosensitive ODN probe is suitable for practical use, we performed a quick single nucleotide polymorphism (SNP) typing as an example. We synthesized ODN (^{BNTV}U), containing a naphthyl triazole moiety and biotin, first synthesizing a naphthyl-azide-containing biotin derivative, and then carrying out a Huisgen cycloaddition between the azide and ODN (^{EV}U). After purification of the products, ODN (^{BNTV}U) was characterized by MALDI-TOF-MS.

We constructed the DNA chip by attaching amino-labeled ODNs onto an aldehyde-modified glass surface. A glass chip spotted with target [ODN (WT) or ODN (MUT) and ODN (^{BNTV}U), 2 μM] was irradiated at 366 nm for 10 min in sodium cacodylate buffer (50 mM, pH 7.0; Figure 3B). After the chip had been washed with deionized water at 98 °C for 5 min, a phosphate-buffered saline (PBS) solution of streptavidin–Cy3 conjugate was added to the surface, and the chip was washed twice in PBS. Fluorescence signals were detected on a microarray scanner. As shown in Figure 3C and D, we measured strong fluorescence signals from the photoligated product in the completely complementary case, showing a measured rate 10^3 times higher than that in the case with a single mismatch in the target sequence.

In the photochemical mismatch detection, there are two possible sources of specificity: the ligation reaction itself and the hybridization selectivity between the target and capture strands. A conventional way of increasing target selectivity is to wash the DNA chip with a buffer solution at a suitable temperature, which results in the dehybridization of DNA duplexes

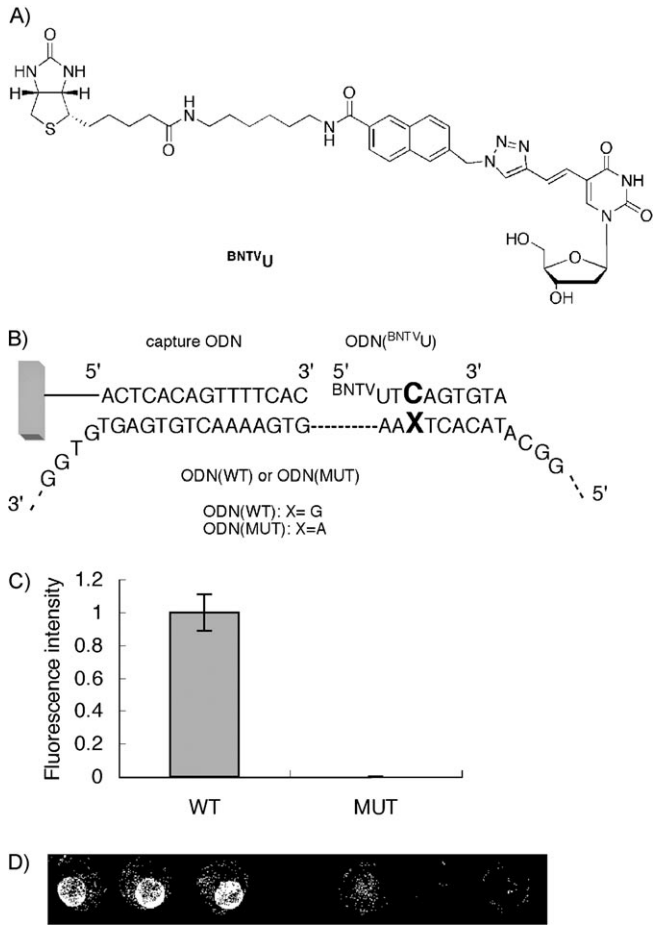

Figure 3. A) Structure of ^{BNTV}U. B) Strategy for the detection of single nucleotide differences on a DNA chip. C) Fluorescence intensity acquired on a microarray scanner for the product of photoligation on matched and singly mismatched target ODNs. D) Fluorescence images.

formed from noncomplementary strands. Mutation detection by the traditional method shows only hybridization specificity, so the loss of DNA duplexes of matched sequences, particularly during the washing step employed to remove noncomplementary strands from the DNA chip, together with incomplete washing out of the mismatched sequences, decreases the selectivity. In contrast, the fluorescence image after use of the photochemical ligation method showed no loss of the biotin-tagged probe strand, due to the covalent bonding between the capture and probe strands. Moreover, mismatched duplexes and other components with the potential to harm the fluorescence imaging were eliminated completely by the high temperatures. Handling of our system is also easier than the traditional method, because our system can achieve selectivity without the annoying requirement for thermal stringency during the photochemical ligation reaction.[3e] Additionally we have already tested the generality of the photochemical SNP typing method with various sequences in our previous works.[3e,23]

In summary, we have synthesized a new photosensitive ODN probe for rapid photoligation through Cu^{I}-catalyzed Huisgen 1,3-dipolar cycloadditions. We have succeeded in performing photoligation on a timescale of seconds. We have also synthesized a photochemical probe for quick SNP typing by Huisgen cycloaddition. Biotin was incorporated with the photochemical probe in this report; however, this method can be employed for various types of labeling, such as spin labeling, which is required in investigation of the structures and dynamics of proteins[24] and nucleic acids[25,26] by electron paramagnetic resonance,[27] and labeling with ferrocene,[28] which is a privileged label for electrochemical detection of biomolecules.[29,30,31] This technique can be applied to upgrade and functionalize the photoligation probe and thus contribute to the development of genetic science.

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Keywords: click chemistry · DNA labeling · DNA · genetic science · photochemistry

[1] M. Nilsson, G. Barbany, D.-O. Antson, K. Gertow, U. Landegren, *Nat. Biotechnol.* **2000**, *18*, 791–793.
[2] R. Favis, J. P. Day, N. P. Gerry, C. Phelan, S. Narod, F. Barany, *Nat. Biotechnol.* **2000**, *18*, 561–564.
[3] a) K. Fujimoto, S. Matsuda, N. Takahashi, I. Saito, *J. Am. Chem. Soc.* **2000**, *122*, 5646–5647; b) K. Fujimoto, S. Matsuda, M. Hayashi, I. Saito, *Tetrahedron Lett.* **2000**, *41*, 7897–7900; c) K. Fujimoto, N. Ogawa, M. Hayashi, S. Matsuda, I. Saito, *Tetrahedron Lett.* **2000**, *41*, 9437–9440; d) Y. Yoshimura, Y. Noguchi, H. Sato, K. Fujimoto, *ChemBioChem* **2006**, *7*, 598–601; e) S. Ogasawara, K. Fujimoto, *Angew. Chem.* **2006**, *118*, 4624–4627; *Angew. Chem. Int. Ed.* **2006**, *45*, 4512–4515.
[4] a) Z. Zhou, C. J. Fahrni, *J. Am. Chem. Soc.* **2004**, *126*, 8862–8863; b) K. Sivakumar, F. Xie, B. M. Cash, S. Long, H. N. Barnhill, Q. Wang, *Org. Lett.* **2004**, *6*, 4603–4606.
[5] a) R. Huisgen, *Angew. Chem.* **1963**, *75*, 604–637; *Angew. Chem. Int. Ed. Engl.* **1963**, *2*, 565–598; b) R. Huisgen, G. Szeimies, L. Möbius, *Chem. Ber.* **1967**, *100*, 2494–2507.
[6] a) H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem.* **2001**, *113*, 2056–2075; *Angew. Chem. Int. Ed.* **2001**, *40*, 2004–2021; b) V. D. Bock, H. Hiemstra, J. H. van Maarseveen, *Eur. J. Org. Chem.* **2006**, 51–68.
[7] C. W. Tornøe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057–3064.
[8] 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.
[9] T. S. Seo, Z. Li, H. Ruparel, J. Ju, *J. Org. Chem.* **2003**, *68*, 609–612.
[10] T. S. Seo, X. Bai, D. H. Kim, Q. Meng, S. Shi, H. Ruparel, Z. Li, N. J. Turro, J. Ju, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 5926–5931.
[11] R. L. Weller, S. R. Rajski, *Org. Lett.* **2005**, *7*, 2141–2144.
[12] G. A. Burley, J. Gierlich, M. R. Mofid, H. Nir, S. Tal, Y. Eichen, T. Carell, *J. Am. Chem. Soc.* **2006**, *128*, 1398–1399.
[13] J. Gierlich, G. A. Burley, P. M. E. Gramlich, D. M. Hammond, T. Carell, *Org. Lett.* **2006**, *8*, 3639–3642.
[14] F. Seela, V. R. Sirivolu, *Chem. Biodiversity* **2006**, *3*, 509–514.
[15] F. Seela, V. R. Sirivolu, *Helv. Chim. Acta* **2007**, *90*, 535–552.
[16] M. Fischler, U. Simon, H. Nir, Y. Eichen, G. A. Burley, J. Gierlich, P. M. E. Gramlich, T. Carell, *Small* **2007**, *3*, 1049–1055.
[17] D. M. Hammond, A. Manetto, J. Gierlich, V. A. Azov, P. M. E. Gramlich, G. A. Burley, M. Maul, T. Carell, *Angew. Chem.* **2007**, *119*, 4262–4265; *Angew. Chem. Int. Ed.* **2007**, *46*, 4184–4187.
[18] I. Géci, V. V. Filichev, E. B. Pedersen, *Chem. Eur. J.* **2007**, *13*, 6379–6386.
[19] C. Bouillon, A. Meyer, S. Vidal, A. Jochum, Y. Chevolot, J.-P. Cloarec, J.-P. Praly, J.-J. Vasseur, F. Morvan, *J. Org. Chem.* **2006**, *71*, 4700–4702.

[20] N. K. Devaraj, G. P. Miller, W. Ebina, B. Kakaradov, J. P. Collman, E. T. Kool, C. E. D. Chidsey, *J. Am. Chem. Soc.* **2005**, *127*, 8600–8601.
[21] R. Kumar, A. El-Sagheer, J. Tumpane, P. Lincoln, L. M. Wilhelmsson, T. Brown, *J. Am. Chem. Soc.* **2007**, *129*, 6859–6864.
[22] K. Barral, A. D. Moorhouse, J. E. Moses, *Org. Lett.* **2007**, *9*, 1809–1811.
[23] a) Y. Yoshimura, D. Okamura, M. Ogino, K. Fujimoto, *Org. Lett.* **2006**, *8*, 5049–5051; b) T. Ami, G. Ozaki, Y. Yoshimura, K. Fujimoto, *Chem. Lett.* **2008**, *37*, 134–135.
[24] W. L. Hubbell, A. Gross, R. Langen, M. A. Lietzow, *Curr. Opin. Struct. Biol.* **1998**, *8*, 649–656.
[25] a) S. Nagahara, A. Murakami, K. Makino, *Nucleosides Nucleotides* **1992**, *11*, 889–901; b) T. E. Edwards, T. M. Okonogi, B. H. Robinson, S. T. Sigurdsson, *J. Am. Chem. Soc.* **2001**, *123*, 1527–1528; c) P. Z. Qin, S. E. Butcher, J. Feigon, W. L. Hubbell, *Biochemistry* **2001**, *40*, 6929–6936.
[26] a) A. Spaltenstein, B. H. Robinson, P. B. Hopkins, *J. Am. Chem. Soc.* **1988**, *110*, 1299–1301; b) P. L. Fischhaber, A. W. Reese, T. Nguyen, J. J. Kirchner, E. J. Hustedt, B. H. Robinson, P. B. Hopkins, *Nucleosides Nucleotides* **1997**, *16*, 365–377; c) P. Z. Qin, K. Hideg, J. Feigon, W. L. Hubbell, *Biochemistry* **2003**, *42*, 6772–6783; d) A. Okamoto, T. Inasaki, I. Saito, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3415–3418; e) O. Schiemann, N. Piton, Y. Mu, G. Stock, J. W. Engels, T. F. Prisner, *J. Am. Chem. Soc.* **2004**, *126*, 5722–5729.
[27] M. Ubbink, J. A. R. Worrall, G. W. Canters, E. J. J. Groenen, M. Huber, *Annu. Rev. Biophys. Biomol. Struct.* **2002**, *31*, 393–422.
[28] D. R. van Staveren, N. Metzler-Nolte, *Chem. Rev.* **2004**, *104*, 5931–5985.
[29] a) C. J. Yu, H. Wang, Y. J. Wan, H. Yowanto, J. C. Kim, L. H. Donilon, C. L. Tao, M. Strong, Y. C. Chong, *J. Org. Chem.* **2001**, *66*, 2937–2942; b) C. J. Yu, Y. J. Wan, H. Yowanto, J. Li, C. L. Tao, M. D. James, C. L. Tan, G. F. Blackburn, T. J. Meade, *J. Am. Chem. Soc.* **2001**, *123*, 11155–11161.
[30] a) C. J. Yu, H. Yowanto, Y. J. Wan, T. J. Meade, Y. Chong, M. Strong, L. H. Donilon, J. F. Kayyem, M. Gozin, G. F. Blackburn, *J. Am. Chem. Soc.* **2000**, *122*, 6767–6768; b) A. R. Pike, L. C. Ryder, B. R. Horrocks, W. Clegg, M. R. J. Elsegood, B. A. Connolly, A. Houlton, *Chem. Eur. J.* **2002**, *8*, 2891–2899.
[31] a) D. A. Di Giusto, W. A. Wlassoff, S. Geisebrecht, J. J. Gooding, G. C. King, *J. Am. Chem. Soc.* **2004**, *126*, 4120–4121; b) D. A. Di Giusto, W. A. Wlassoff, S. Geisebrecht, J. J. Gooding, G. C. King, *Angew. Chem.* **2004**, *116*, 2869–2872; *Angew. Chem. Int. Ed.* **2004**, *43*, 2809–2812; c) W. A. Wlassoff, G. C. King, *Nucleic Acids Res.* **2002**, *30*, e58; d) S. S. W. Yeung, T. M. H. Lee, I.-M. Hsing, *J. Am. Chem. Soc.* **2006**, *128*, 13374–13375; e) F. Patolsky, Y. Weizmann, I. Willner, *J. Am. Chem. Soc.* **2002**, *124*, 770–772.

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