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Synthesis and base-pairing properties of C-nucleotides having 1-substituted 1*H*-1,2,3-triazoles

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ABSTRACT

Oligonucleotides including C-nucleotides having 1-substitued 1*H*-1,2,3-triazoles as artificial nucleobases were conveniently synthesized by the post-elongation modification method using the copper(I)-catalyzed alkyne–azide 1,3-dipolar cycloaddition (CuAAC) reaction. The base-pairing properties of the triazole nucleobase analogs in forming duplexes with oligonucleotides were investigated by the T_m experiments.

Chemically modified oligonucleotides are currently attracting much attention because of their applications as potent tools for molecular biology, as diagnostic probes and/or as potential materials for oligonucleotide-based therapy.[1] In particular, modification of a nucleobase moiety is widely used to increase base-discrimination ability and to enhance the stability of duplex or triplex nucleic acids.[1–5] These properties of base-modified oligonucleotides are very important and useful for their application to many oligonucleotide-based technologies.

The copper(I)-catalyzed alkyne–azide 1,3-dipolar cycloaddition (CuAAC),[6–9] giving a 1,2,3-triazole derivative, has been extensively studied in both organic chemistry and chemical biology. For example, syntheses of biomolecule-functional molecule conjugates,[10–13] novel bioactive compounds,[14,15] and circular oligonucleotides[16–18] have been achieved by CuAAC. Thus, to develop a new class of base-modified oligonucleotides, we considered that employing a post-elongation modification method with CuAAC would produce 1-substituted 1*H*-1,2,3-triazole nucleobase analogs (Scheme 1).

Here, we demonstrate the synthesis of an oligonucleotide containing 1-ethynyl-2-deoxy-β-D-ribofuranose, and the efficient conversion of the ethynyl group into several 1,2,3-triazoles, thereby producing novel nucleobase analogs. Moreover, the duplex-forming ability of the obtained oligonucleotides containing triazole C-nucleotides with ssDNA is evaluated by T_m experiments.

Synthesis of the phosphoramidite derivative **1**β was achieved as shown in Scheme 2. As previously reported,[14] **2** was prepared as an anomeric mixture (α:β = ca. 3:1),[19] which was then treated with sodium methoxide in MeOH to give **3**.[20] After protection of the primary hydroxyl group of **3** with dimethoxytrityl (DMTr),[21] each anomer was readily separated by silica gel chromatography. Phosphitylation reactions on **4α** and **4β** provided the desired phosphoramidites **1**α and **1**β in 76% and 69% yields, respectively.[22] Phosphoramidite **1**β was then incorporated into a 15-mer homopyrimidine oligonucleotide on an automated DNA synthesizer using a standard phosphoramidite protocol.[23] Using a trityl monitor, the coupling efficiency of **1**β was estimated to be >99%. The obtained oligonucleotide **ON-1** was purified by RP-HPLC and its composition was confirmed by MALDI-TOF-MS.

Next, **ON-1** was reacted with benzylazide to determine the optimal conditions for CuAAC (Scheme 3). The conversion efficiency was evaluated by RP-HPLC analysis (Fig. 1). Under condition A [copper(II) sulfate (2 equiv), sodium ascorbate (2 equiv), benzylazide (2.2 equiv) in 10% THF (aq) buffer], the reaction proceeded moderately at room temperature, and ca. 50% conversion to **ON-2** was observed after 15 h (Fig. 1a). The reaction almost went to completion during the same period of time under condition B [copper(II) sulfate (4 equiv), sodium ascorbate (4 equiv), benzylazide (5 equiv) in 10% THF (aq) buffer] (Fig. 1b). As previously reported,[9] tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (TBTA) effectively promoted CuAAC in our experiments. After several attempts, we found that the reaction went to completion within 90 min under condition C [copper(II) sulfate (2 equiv), sodium ascorbate (4 equiv), TBTA (4 equiv), benzylazide (10 equiv) in 30% DMSO (aq) buffer] (Fig. 1c and Table 1, entry 1).

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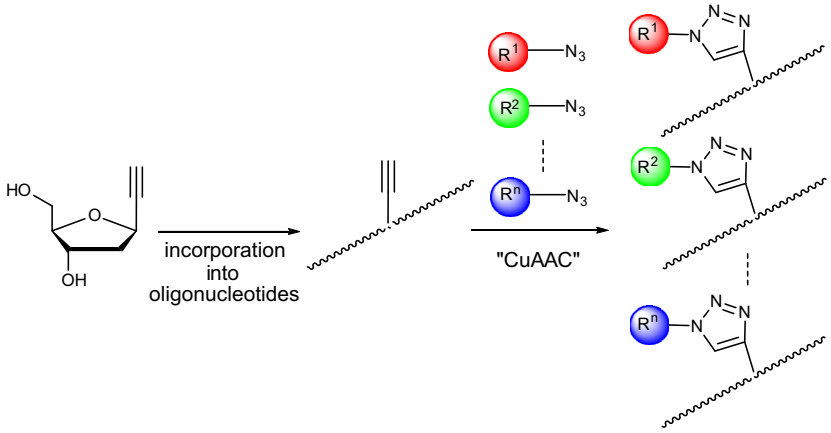
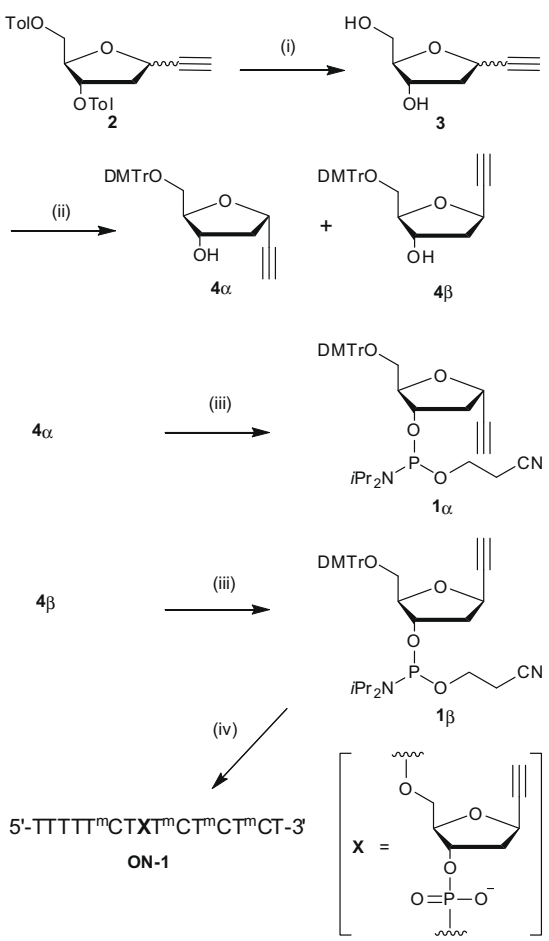
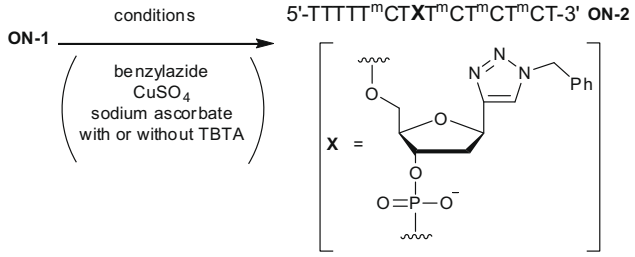


Scheme 1. Schematic representation of the synthesis of oligonucleotides bearing 1-substituted 1*H*-1,2,3-triazole nucleobase analogs by post-elongation modification methods using CuAAC.



Scheme 2. Synthesis of phosphoramidite **1** and incorporation into an oligonucleotide. Reagents and conditions: (i) NaOMe, MeOH, rt, 3 h, 92%; (ii) dimethoxytrityl chloride, pyridine, rt, 2 h, 56% for **4α** and 18% for **4β**; (iii) $(i\mathrm{Pr}_2\mathrm{N})_2\mathrm{PO}(\mathrm{CH}_2)_2\mathrm{CN}$, diisopropylammonium tetrazolide, MeCN/THF = 3:1, rt, 4 h, 76% for **1α** and 69% for **1β**; (iv) automated DNA synthesizer. In the **ON-1** sequence, ^{m}C stands for 2′-deoxy-5-methylcytidine.

Following optimization of the reaction conditions for CuAAC between **ON-1** and benzylazide, we evaluated the reaction of **ON-1** with several other azide compounds (Table 1 and Scheme 4). In addition to benzylazide (entry 1), primary azides (entries 2 and 3), a secondary azide (entry 4), a tertiary azide (entry 5) and aro-



Scheme 3. Conversion of **ON-1** to **ON-2** by CuAAC with benzylazide.

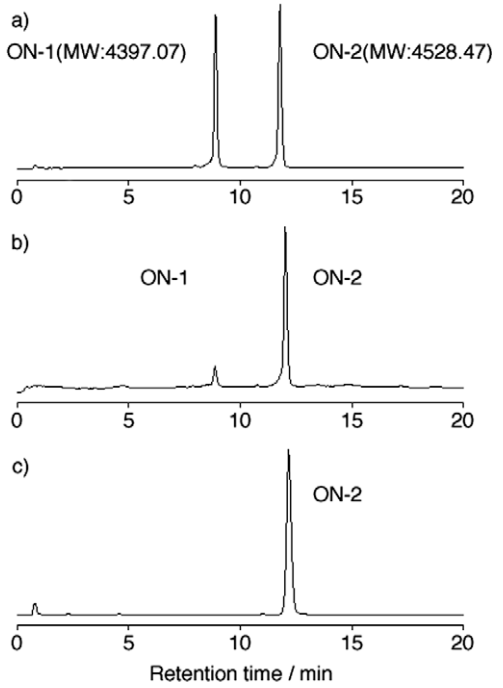
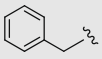
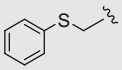
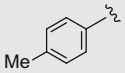
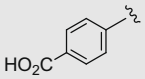
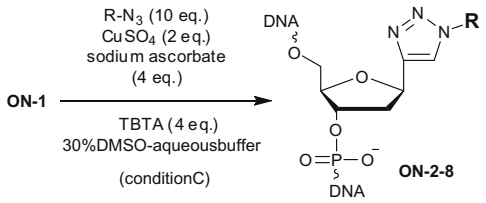


Figure 1. HPLC analysis of CuAAC between **ON-1** and benzylazide. The reaction was carried out at room temperature under (a) condition A [copper(II) sulfate (2 equiv), sodium ascorbate (2 equiv), benzylazide (2.2 equiv) in 10% THF (aq) buffer] for 15 h; (b) condition B [copper(II) sulfate (4 equiv), sodium ascorbate (4 equiv), benzylazide (5 equiv) in 10% THF (aq) buffer] for 15 h and (c) condition C [copper(II) sulfate (2 equiv), sodium ascorbate (4 equiv), TBTA (4 equiv), benzylazide (10 equiv) in 30% DMSO (aq) buffer] for 90 min. The peaks at 8.9 and 12.0 min correspond to **ON-1** (reactant) and **ON-2** (product), respectively. The samples for the corresponding peaks were collected and characterized by MALDI-TOF-MS. HPLC conditions: reversed phase HPLC (Waters Xterra RP column) with acetonitrile/water containing 100 mM triethylamine-acetic acid (TEAA) buffer (pH 7.0) as mobile phase, linear gradient 8–20% acetonitrile/water (30 min, 1.0 mL/min).

Table 1
MALDI-TOF-MS data and yields of the oligonucleotides obtained via Scheme 4[a]

Entry	R	Obtained oligonucleotides		
		MALDI-TOF-MS data		Conversion efficiency[b] (%)
		Calcd. $[M-H]^-$	Found $[M-H]^-$	
1	 (**ON-2**)	4529.06	4528.47	98
2	 (**ON-3**)	4595.20	4596.19	100
3	 (**ON-4**)	4561.12	4562.37	97
4	 (**ON-5**)	4521.08	4522.96	91
5	 (**ON-6**)	4573.15	4573.22	38
6	 (**ON-7**)	4529.06	4531.03	79
7	 (**ON-8**)	4559.04	4557.79	81

[a] The reaction was conducted for 90 min at room temperature under condition C [copper(II) sulfate (2 equiv), sodium ascorbate (4 equiv), TBTA (4 equiv), azide compound (10 equiv) in 30% DMSO (aq) buffer].
[b] The conversion efficiency was evaluated by RP-HPLC analysis from the peak areas of **ON-1** and the obtained oligonucleotide.



Scheme 4. CuAAC reaction of **ON-1** with several azide reagents.

matic azides (entries 6 and 7) were treated with **ON-1** using condition C at room temperature for 90 min. Although the reaction with a tertiary azide (entry 5) afforded **ON-6** in only moderate yield, probably due to steric hindrance of the tertiary azide, most reactions proceeded smoothly, and the desired oligonucleotides **ON-2–5**, **ON-7** and **ON-8** bearing the corresponding 1-substituted 1*H*-1,2,3-triazoles as an artificial nucleobase were successfully obtained (entries 1–4, 6 and 7).

Finally, the duplex-forming ability of the oligonucleotides **ON-2–8** containing triazole nucleobase analogs with ssDNA, 5′-AGAGAGAYAGAAAAA-3′ (Y = A, G, T or C), was examined by T_m experiments and compared with those of ethynyl derivative **ON-1** and natural **ON-9**. The T_m values are summarized in Table 2. In general, ON-**2**–**8** stabilized the duplex better than **ON-1**, presumably due to the stacking effect of the triazole nucleobases, while duplexes of **ON-2–8** with ssDNA targets (Y = A, G, T and C) were less stable than the full-match one comprising **ON-9** and ssDNA (Y = A). **ON-2–8** were also found to form the most stable duplex with ssDNA (Y = G) among all ssDNA targets, indicating that a nitrogen atom in the triazole structure can make a hydrogen bond with the 2-NH_2 or 1-NH group of G. In comparison with benzyl-substituted **ON-2**, **ON-4** bearing a (phenylthio)methyl group was favorable for duplex formation, and it would be because the hydrophobicity of a nucleobase moiety increased by an additional sulfur atom. Interestingly, **ON-4** had almost the same stability against all ssDNA targets (the T_m values ranged from 37 °C to 40 °C). This suggests that 1-(phenylthio)methyl-1*H*-1,2,3-triazole is a new candidate as a non-discriminatory nucleobase, namely a universal base,[24] though more minute examination is naturally required. Results of **ON-5** and **ON-7** demonstrated that an aromatic ring at the 1-position of a triazole moiety increased the duplex stability, probably due to the stacking interaction with the neighboring base-pairs. **ON-8** with a carboxyl group destabilized the duplex compared to **ON-7** with a methyl group at the same position, indicating that the hydrophilic group in that position was unsuitable for duplex formation. In addition, it should be noted that **ON-6** showed du-

Table 2
T_m values (°C) for duplex between **ON-1–9** and ssDNA targets[a]

Oligonucleotides	Target ssDNA (5′-AGAGAGAYAGAAAAA-3′)			
	Y = A	Y = G	Y = T	Y = C
ON-1	32	32	31	29
ON-2	32	38	33	34
ON-3	35	39	39	39
ON-4	37	40	40	38
ON-5	34	37	33	34
ON-6	30	37	34	34
ON-7	39	42	38	36
ON-8	33	38	33	33
ON-9[b]	52	42	42	39

[a] The measurement was carried out under 10 mM phosphate buffer (pH 7.0), 100 mM NaCl and 3 μM of each oligonucleotide.
[b] The sequence is 5′-TTTTT^{m}CTTT^{m}CT^{m}CT^{m}CT-3′.

plex formation even though it has a bulky adamantyl group on the nucleobase moiety.

In conclusion, we achieved the synthesis of oligonucleotides including C-nucleotides having 1-substituted 1*H*-1,2,3-triazoles as artificial nucleobases by the post-elongation modification method using the CuAAC reaction between a 1-ethynyl-2-deoxy-β-D-ribofuranose moiety in an oligonucleotide and several azide compounds. In light of its simplicity and versatility, this method would be quite useful for finding new 1,2,3-triazole-based nucleobase having distinguished functions. Moreover, the T_m experiments of the obtained oligonucleotide derivatives showed that 1-(phenylthio)methyl-1*H*-1,2,3-triazole could act as a universal base. Thus, this nucleobase analog may be used as an ambiguous site in primers for PCR and sequencing.[24] Currently, further investigation on this potential universal nucleobase is in progress.

Acknowledgments

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References and notes

1. *Methods in Molecular Biology*; Herdewijn, P., Ed.Oligonucleotide Synthesis: Methods and Applications; Human Press Inc: Totowa, NJ, 2005; Vol. 288,.
2. For reviews, see: (a) Buchini, S.; Leumann, C. J. *Curr. Opin. Chem. Biol.* **2003**, *7*, 717; (b) Luyten, I.; Herdewijn, P. *Eur. J. Med. Chem.* **1998**, *33*, 515.
3. *Triple Helix Forming Oligonucleotides*; Malvy, C., Harel-Bellan, A., Pritchard, L. L., Eds.; Kluwer Academic: Boston, 1999.
4. Guianvarc'h, D.; Fourrey, J. L.; Maurisse, R.; Sun, J. S.; Benhida, R. *Bioorg. Med. Chem.* **2003**, *11*, 2751.
5. (a) Obika, S.; Hari, Y.; Morio, K.; Imanishi, T. *Tetrahedron Lett.* **2000**, *41*, 221; (b) Obika, S.; Hari, Y.; Sekiguchi, M.; Imanishi, T. *Angew. Chem., Int. Ed.* **2001**, *40*, 2079. *Angew. Chem.* **2001**, *113*, 2149; (c) Obika, S.; Hari, Y.; Sekiguchi, M.; Imanishi, T. *Chem. Eur. J.* **2002**, *8*, 4796; (d) Hari, Y.; Obika, S.; Sakaki, M.; Morio, K.; Yamagata, Y.; Imanishi, T. *Tetrahedron* **2002**, *58*, 3051; (e) Hari, Y.; Obika, S.; Sekiguchi, M.; Imanishi, T. *Tetrahedron* **2003**, *59*, 5123; (f) Hari, Y.; Obika, S.; Inohara, H.; Ikejiri, M.; Une, D.; Imanishi, T. . *Chem. Pharm. Bull.* **2005**, *53*, 843; (g) Obika, S.; Inohara, H.; Hari, Y.; Imanishi, T. *Bioorg. Med. Chem.* **2008**, *16*, 2945.
6. For recent reviews, see: (a) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004; (b) Kolb, H. C.; Sharpless, K. B. *Drug Discovery Today* **2003**, *8*, 1128.
7. Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057.
8. Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596.
9. Chan, T. R.; Hilgraf, R.; Sharpless, K. B.; Fokin, V. V. *Org. Lett.* **2004**, *6*, 2853.
10. Wolfbeis, O. S. *Angew. Chem., Int. Ed.* **2007**, *46*, 2980.
11. (a) Geci, I.; Filichev, V. V.; Pedersen, E. B. *Chem. Eur. J.* **2007**, *13*, 6379; (b) Kumar, R.; El-Sagheer, A.; Tumpane, J.; Lincoln, P.; Wilhelmsson, L. M.; Brown, T. *J. Am. Chem. Soc.* **2007**, *129*, 6859.
12. Manetsch, R.; Krasinski, A.; Radic, Z.; Raushel, J.; Taylor, P.; Sharpless, K. B.; Kolb, H. C. *J. Am. Chem. Soc.* **2004**, *126*, 12809.
13. Zhang, Y. H.; Gao, Z. X.; Zhong, C. L.; Zhou, H. B.; Chen, L.; Wu, W. M.; Peng, X. J.; Yao, Z. J. *Tetrahedron* **2007**, *63*, 6813.
14. Wamhoff, H.; Warnecke, H. *Arkivoc* **2001**, 95.
15. Zhu, R.; Wang, M.; Xia, Y.; Qu, F. Q.; Neyts, J.; Peng, L. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3321.
16. Nakane, M.; Ichikawa, S.; Matsuda, A. *Nucleic Acids Symp. Ser.* **2005**, *49*, 189.
17. Lietard, J.; Meyer, A.; Vasseur, J. J.; Morvan, F. *J. Org. Chem.* **2008**, *73*, 191.
18. Nakane, M.; Ichikawa, S.; Matsuda, A. *J. Org. Chem.* **2008**, *73*, 1842.
19. In Ref. 14, only the α-anomer of **2** was isolated and employed for alkyne-azide 1,3-dipolar cycloaddition.
20. Recently, a similar synthetic procedure for compound **3** (anomeric mixture) was reported, see: Heinrich, D.; Wagner, T.; Diederichsen, U. *Org. Lett.* **2007**, *9*, 5311.
21. Compound **4β** was also synthesized by an alternative route, see: Chiba, J.; Takeshima, S.; Mishima, K.; Maeda, H.; Nanai, Y.; Mizuno, K.; Inouye, M. *Chem. Eur. J.* **2007**, *13*, 8124.
22. *Selected data of* **1α** and **1β**. **1α**: ^{31}P NMR ($CDCl_3$) δ 147.9, 148.3; HRMS (FAB) *m/z* calcd for $C_{37}H_{45}N_2NaO_6P$ ($M+Na^+$): 667.2913; found 667.2909. **1β**: ^{31}P NMR ($CDCl_3$) δ 148.5, 149.1; HRMS (FAB) *m/z* calcd for $C_{37}H_{45}N_2NaO_6P$ ($M+Na^+$): 667.2913; found 667.2915.
23. The phosphoramidite **1α** was also successfully incorporated into an oligonucleotide on an automated DNA synthesizer.
24. For a review, see: Loakes, D. *Nucleic Acids Res.* **2001**, *29*, 2437.