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

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Oligonucleotides including C-nucleotides having 1-substituted 1H-1,2,3-triazoles as artificial nucleobases were conveniently synthesized by the post-elongation modification method using the copper(I)-catalyzed alkynyl–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 Tm experiments.

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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 triple nucleic acids.1–5 These properties of base-modified oligonucleotides are very important and useful for their application to many oligonucleotide- and nucleobase-based technologies.

The copper(I)-catalyzed alkynyl–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 conjugates10–13 novel bioactive compounds,14,15 and circular oligonucleotides16–18 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 1H-1,2,3-triazole nucleobase analogs (Scheme 1).

Here, we demonstrate the synthesis of an oligonucleotide containing 1-ethyl-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 Tm experiments.

Synthesis of the phosphoramidite derivative 1β was achieved as shown in Scheme 2. As previously reported,14 2 was prepared as an anomic 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 1α and 1β in 76% and 69% yields, respectively.22 Phosphoramidite 1β 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 1β 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-1H-1,2,3-triazol-4-yl]methylamine (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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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 1. Schematic representation of the synthesis of oligonucleotides bearing 1-substituted 1H-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) (iPr₂N)₂PO(CH₂)₂CN, 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, 5°C stands for 2’-deoxy-5-methylcytidine.

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) in 10% THF (aq) buffer] for 15 h; (b) condition B [copper(II) sulfate (4 equiv), sodium ascorbate (4 equiv), benzylazide (2.2 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 triethylammonium-acetic acid (TEAA) buffer (pH 7.0) as mobile phase, linear gradient 8–20% acetonitrile/water (30 min, 1.0 mL/min).
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 1H-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’-AGAGAGAGAGAGAGAGAGAG-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-NH2 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-1H-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-po-

### Table 1
MALDI-TOF-MS data and yields of the oligonucleotides obtained via Scheme 4*

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>Calculated MALDI-TOF-MS data (M–H)</th>
<th>Found MALDI-TOF-MS data (M–H)</th>
<th>Conversion efficiencya (%)</th>
</tr>
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<tr>
<td>1</td>
<td></td>
<td>4529.06</td>
<td>4528.47</td>
<td>98</td>
</tr>
<tr>
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<td>4595.20</td>
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<td>100</td>
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<tr>
<td>7</td>
<td>HO2C</td>
<td>4559.04</td>
<td>4557.79</td>
<td>81</td>
</tr>
</tbody>
</table>

* 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.

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![Scheme 4](image-url)
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 1H-1,2,3-triazoles as artificial nucleobases by the post-elongation modification method using the CuAAC reaction between a 1-ethyl-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-1H-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. Currently, further investigation on this potential universal nucleobase is in progress.

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References and notes

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.
22. Selected data of 1a and 1b. 1H NMR (CDCl3) \( \delta \) 147.9, 148.3; HRMS (FAB) m/z calcd for \( C_{27}H_{18}N_2NaOP \) (M+Na\(^+\)) 667.2913; found 667.2909. 1H NMR (CDCl3) \( \delta \) 148.5, 149.1; HRMS (FAB) m/z calcd for \( C_{27}H_{18}N_2NaOP \) (M-HA\(^-\)) 667.2913; found 667.2915.
23. The phosphoramidate 1b was also successfully incorporated into an oligonucleotide on an automated DNA synthesizer.