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Triazole-Linked Analogue of Deoxyribonucleic Acid (^{TL}DNA): Design, Synthesis, and Double-Strand Formation with Natural DNA

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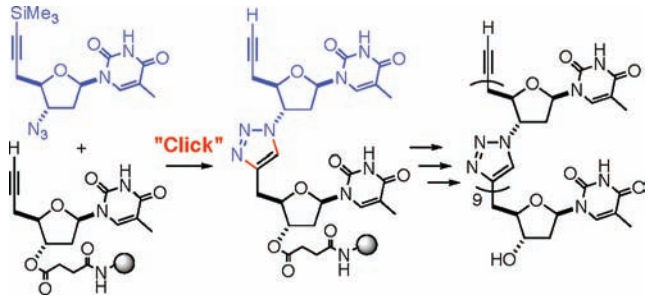
ABSTRACT



A new triazole-linked analogue of DNA (^{TL}DNA) has been designed and synthesized using click chemistry. The chain elongation reaction using copper-catalyzed Huisgen cycloaddition was successful and gave the artificial oligonucleotide that formed a stable double strand with the complementary strand of natural DNA.

Analogues of DNA that have nucleobases displayed on artificial backbones are attracting much interest. Analogues that can be polymerized by bond-forming reactions other than P−O bond formation are especially important,[1] and peptide nucleic acid (PNA) has emerged as one of the most successful examples among such analogues that form stable multiple strands with complementary DNA.[2] The success of PNA is backed up by the strong synthetic background of carbonyl/peptide chemistry, which highlights the stringent requirement needed for the bond-forming reaction of the elongation process. Thus, an ideal reaction for elongation should give high yield, generate inoffensive byproducts, and proceed under simple reaction conditions. We conjectured that a copper-catalyzed version of the Huisgen [3 + 2] cycloaddition would satisfy such conditions[3−6] and thus designed a triazole-linked analogue of DNA (^{TL}DNA), as shown in Figure 1. Herein, we report on the synthesis of

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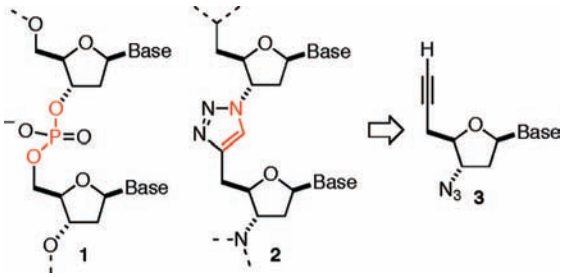


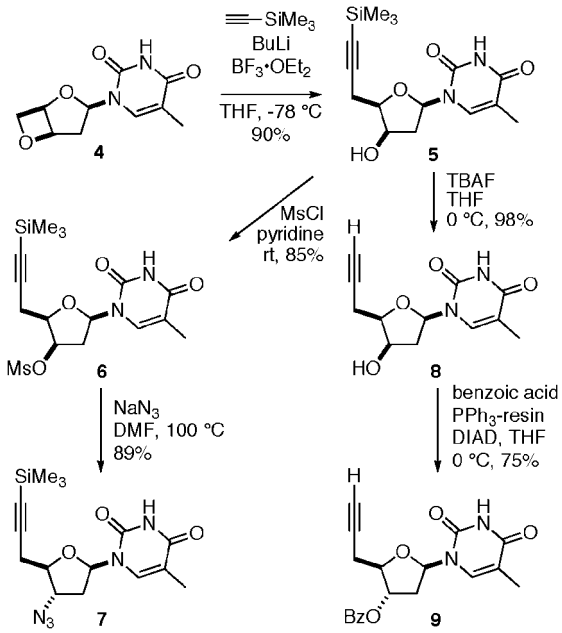
Figure 1. Structure of natural DNA (**1**) and a triazole-linked analogue (^{TL}DNA, **2**) with a retrosynthetic analysis to the monomer (**3**). The natural phosphate linkage, O−P−O, was replaced by a triazole linkage, N−C−C, shown in red.

oligothymine ^{TL}DNA and present the initial results on double-strand formation with a complementary DNA strand.

The molecular design of ^{TL}DNA commenced with the replacement of the phosphodiester linker of natural DNA by a triazole ring (Figure 1). We kept the methylene bridge at pseudo-5′-position to maintain the six-bond periodicity of the oligonucleotides[7] as well as the flexibility of the oligonucleotide chains. We expect that the presence of the sugar-like ring may also be advantageous in retaining any puckering flexibility similar to that of natural DNA. Therefore, the monomer for the elongation reaction should feature an acetylene group at the pseudo-5′-position and an azide function at the pseudo-3′-position (i.e., compound **3** in Figure 1).

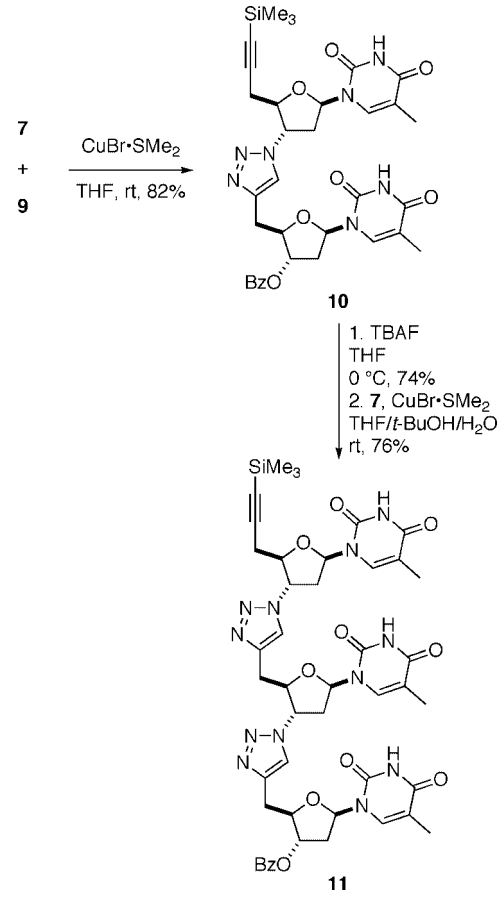
Monomers for ^{TL}DNA were readily accessed from naturally occurring thymidine. Thus, the oxetane compound **4**, derived from thymidine using a reported method,[8] was converted to the acetylene derivative **5** by a Lewis acid mediated nucleophilic ring opening reaction with lithium acetylide (Scheme 1, see the Supporting Information for the experimental details).[9] After the mesylation of **5**, the desired azide **7** bearing a trimethylsilyl-protected acetylene moiety was obtained by nucleophilic substitution of the mesylate **6** using sodium azide.[10] Another monomer **9** for the terminus was prepared from the common intermediate **5** through desilylation and Mitsunobu substitution reactions. All the reactions proceeded to give moderate to good yield and were scalable to multigram-scale synthesis.

Scheme 1. Synthesis of Monomers of ^{TL}DNA



With monomers **7** and **9** in hand, we began examining the coupling reaction in solution. After screening several parameters, such as the solvent and copper reagents, we found that the dimethyl sulfide complex of copper bromide was suited for the Huisgen [3 + 2] cycloaddition, and obtained 2-mer ^{TL}DNA **10** in 82% yield after a reaction time of 15 h in THF (Scheme 2). The efficacy of the reaction for the elongation process was demonstrated by the fact that the reaction did not require an excess of the substrate, in contrast to the need for an excess of the elongating phosphoramidite monomer in a standard DNA synthesis.[11,12] A popular catalyst, a combination of $CuSO_4$ and ascorbate,[5] was also effective for the elongation process, but the reaction did not proceed to completion even after a reaction period of a few days. The ensuing desilylation of **10** with TBAF gave the

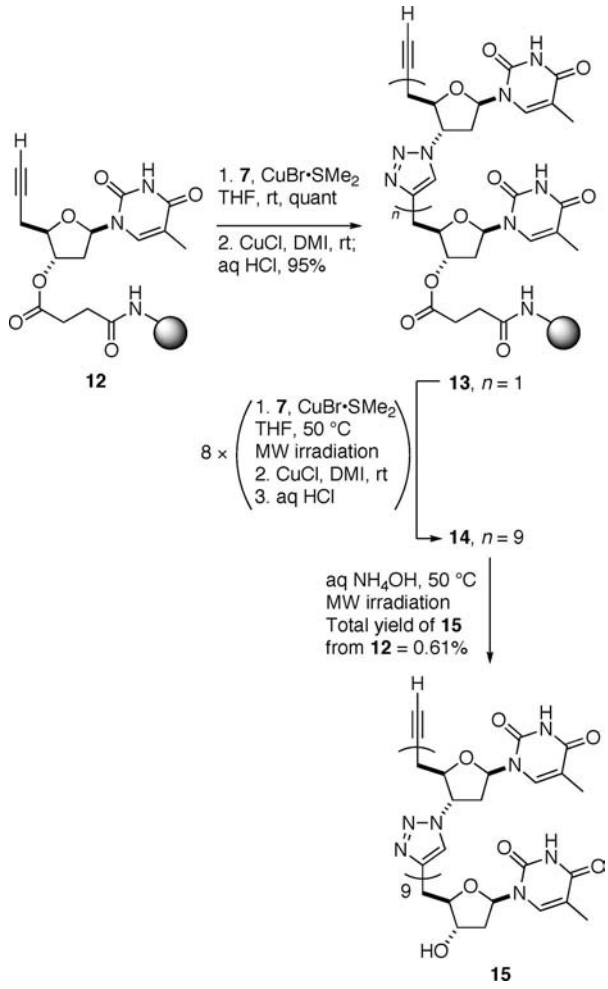
Scheme 2. Solution-Phase Synthesis of the 3-mer ^{TL}DNA



2-mer ^{TL}DNA that was used in the next coupling reaction. Due to the low solubility of the 2-mer ^{TL}DNA in THF, the second elongation reaction with the azide **7** was carried out in a polar solvent and gave 3-mer ^{TL}DNA **11** in 76% yield when the reaction was carried out in an aqueous mixture (THF/*t*-BuOH/H_2O).

With the aim of producing longer oligomers, we then examined a solid-phase synthesis and succeeded in synthesizing the 10-mer ^{TL}DNA. We reoptimized the reaction conditions for elongation and deprotection on a solid support for the synthesis of 2-mer ^{TL}DNA. The reaction was carried out with the terminal monomer **12** loaded on a poly(ethylene glycol)-polystyrene-based resin (NovaSyn TG amino resin, 0.27 mmol/g; Scheme 3), and the yield was estimated after cleavage from the resin. Thus, the first coupling reaction proceeded smoothly under conditions similar to those of the solution-phase reaction, and the 2-mer ^{TL}DNA was obtained quantitatively. However, the subsequent desilylation was not successful using TBAF because the ^{TL}DNA cleaved from the resin under the nucleophilic conditions. Then we found that a copper-mediated desilylation proceeded smoothly to give the unprotected acetylene derivative **13** without a cleavage reaction taking place.[13] Although the elongation reaction with **13** on a solid support proceeded in an aqueous solvent (THF/*t*-BuOH/H_2O), we found that microwave irradiation dramatically accelerated the reaction in THF.[6] Thus, the 3-mer ^{TL}DNA was obtained in 84% yield from the reaction carried out in THF under microwave irradiation for a period of 1.5 h. Having finalized the reaction conditions on a solid support, we repeated the deprotection/elongation reactions and obtained the 10-mer ^{TL}DNA **15** after HPLC purification on a triazole-capped hydrophilic interaction chromatography (HILIC) column (isolated yield = 0.61%, 19 steps). Improvement of the reaction and purification conditions would increase the yield of the final product (see the Supporting Information for details). The polythymine ^{TL}DNA **15** was soluble in water up to ca. 7 μM in water, and the solubility increases in the presence of polar organic solvents such as acetonitrile. Implementation of hydrophilic residue such as polyethylene glycol through click chemistry may increase the solubility in water and thus expand the scope of ^{TL}DNA.

Scheme 3. Solid-Phase Synthesis of 10-mer ^{TL}DNA



Finally, we found that ^{TL}DNA formed a stable double strand with a natural DNA strand. The formation of the double strand was first confirmed by monitoring the hypochromic effect from the base stacking using UV−visible spectroscopy.[14,15] The hypochromic effect at 260 nm was observed when ^{TL}DNA **15** was hybridized with the natural target $d(T)_2(A)_{10}(T)_2$ in SSPE buffer (100 mM sodium chloride, 10 mM sodium phosphate, and 0.10 mM ethylenediamine tetraacetic acid (EDTA), pH 7). The stoichiometric ratio of each strand in the hybridized complex was determined to be 1:1 using the Job plot of the hypochromic change (Figure S1, Supporting Information).[16] The melting temperature (T_m) was much higher (61.1 °C) than that of a control natural DNA $d(T)_{10}$ (20.0 °C), which shows that the analogue bound to the target strand more tightly than the natural DNA. This stability may be due to the neutral backbone that does not have any repulsive interactions with the anionic phosphate backbone of the natural target.[2] Considering the fact that a similar triazole linker with a longer methylene bridge destabilizes the double strand,[17,18] we think that the six-bond periodicity of ^{TL}DNA is a crucial design for the double strand formation. The structural complementarity of ^{TL}DNA for natural DNA is visible in the molecular model (Figure 2b).

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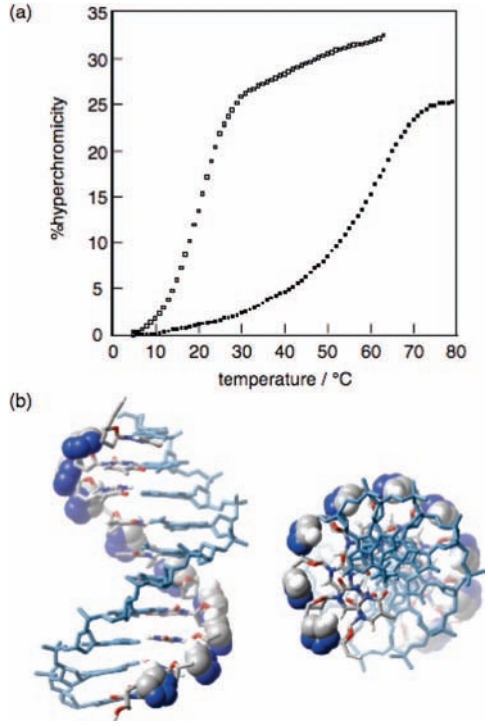

Figure 2. Double-strand formation of ^{TL}DNA with a natural complementary strand. (a) Melting curves of the 10-mer double strands. Filled circle: ^{TL}DNA **15** with natural DNA ($d(T)_2(A)_{10}(T)_2$). Open square: natural DNA $d(T)_{10}$ with $d(T)_2(A)_{10}(T)_2$. (b) Molecular models of the double strand between **15** and $d(A)_{10}$ showing the structural complementarity of two strands (left: side view, right: top view). The triazole linkers are shown in CPK models (Gray: C, red: O, white: H, blue: N), and $d(A)_{10}$ strand are shown in light blue. Top view shows the periodicity of the artificial triazole linkers matches well with that of natural phosphate linkers. Details of the molecular simulation are described in the Supporting Information.

The helical structure of the double strand was experimentally confirmed by the CD spectra (Figure S2, Supporting Information).[15,19] Analysis of the temperature-dependent change of the CD spectra also confirmed the high T_m value (Figure S3, Supporting Information). Investigations on the structural details of the new double helix, for instance, by thermodynamic analysis,[15] are now underway and will be reported in the near future.

In summary, we have reported on the synthesis of a new analogue of DNA using a highly efficient and selective route that should be amenable to large-scale preparation of longer oligonucleotides. The cycloaddition way, examined for the first time for chain elongation, has proven useful for the synthesis of oligomers. As click chemistry tolerates a wide range of functionalities,[5] this method provides a powerful strategy for synthesizing artificial oligonucleotides, including various types of conjugates, and will contribute to the growing scope of oligonucleotide chemistry.[20−26] The tolerance of the reaction for other nucleobases has already been demonstrated previously,[20] and the variation of the nucleobase in future will expand the scope of ^{TL}DNA. Structural features of the new analogue, such as the rigid π-rich backbone or metal-coordination of the triazole rings may also flourish the structural/supramolecular chemistry of oligonucleotides.

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

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