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Convergent synthesis of oligomers of triazole-linked DNA analogue (^{TL}DNA) in solution phase

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ABSTRACT

A convergent route for the solution-phase synthesis of oligomeric triazole-linked analogues of DNA (^{TL}DNA) has been developed. A one-pot procedure for desilylation of masked acetylene and the ensuing copper-catalyzed Huisgen coupling reaction between oligomers allowed the solution-phase synthesis of 7-mer and 8-mer ^{TL}DNA in good yield.

Owning to the interest in the application of oligonucleotides especially in abiological fields such as materials science or supramolecular chemistry,[1] the importance of reliable synthetic methods for the large-scale preparation is increasing. We have recently reported a new oligonucleotide analogue, triazole-linked analogue of DNA (^{TL}DNA), that forms a stable duplex with a natural complementary strand.[2,3] Although the synthesis of the polythymine oligomer utilized one of the most successful reactions in click chemistry, the copper-catalyzed Huisgen cycloaddition,[4] the method was not easily applicable to the large-scale preparation due to the linear synthetic route based on solid-phase synthesis. We herein report on the development of a convergent route to the polythymine oligomers via solution-phase synthesis. The method including optimized conditions for a successive desilylation and click coupling provided scalable synthetic route to 7-mer and 8-mer ^{TL}DNA oligomers.

We first investigated a one-pot procedure for successive deprotection and coupling reactions to minimize the purification steps that often lower the efficiency of the synthesis of oligomers. The successive reactions were investigated for the synthesis of 2-mer ^{TL}DNA, and we found appropriate conditions for the deprotection of silylacetylene moiety in the combination with the following click coupling reaction without interim workup or purification. Thus, a thymidine analogue **1** bearing a trimethylsilylacetylene moiety was treated with desilylation reagents, and an equimolar amount of azide **3** and a copper catalyst were added to the mixture after the consumption of the masked acetylene (Table 1, See Supplementary data for details.). All the desilylation reagents, silver(I) salt,[5] tetrabutylammonium fluoride (TBAF) and H_2O/triethylamine, were tolerable for the click coupling reaction and successfully afforded 2-mer ^{TL}DNA **4** in good yields (entries 1–3). The reactions in aqueous triethylamine required a long reaction time of 24 h (entry 3), but we found that the reaction time was shortened to 3 h in total under microwave irradiation conditions (entry 4).[6] Among the desilylation reagents, however, we found that the silver(I) reagent is not suited for the synthesis of oligomers, as the workup required repeated extractions of the target compound from the aqueous phase probably due to the complexation with silver salt.

We then prepared 3-mer and 4-mer ^{TL}DNA **6** as the 5′-terminus oligomers by converting the 3′-hydroxyl group to an azido group (Scheme 1). Thus, 3′-hydroxyl group of 3-mer **5a** was converted to azido group through mesylation and nucleophilic azidonation in 75% yield, and the following removal of 4,4′-dimethoxytrityl (DMTr) group afforded 3′-azido 3-mer **6a** in 97% yield. A longer 4-mer azide **6b** was also synthesized by the same transformation from **5b**.

Finally, the one-pot procedure was applied to the synthesis of 7-mer **8a** and 8-mer **8b**, respectively (Scheme 2). Thus, 3′-terminus 4-mer **7** was submitted to the successive desilylation and coupling reaction with 5′-terminus 3-mer **6a** under the optimized conditions. As shown in Figure 1 for the desilylation with aqueous triethylamine, the reaction completed within 3 h, and the following coupling reaction afforded the 7-mer **8a**. After the removal of solvent in vacuo and washing with aqueous ammonium chloride, the oligomer **8a** was obtained in 94% yield. Although the reaction with

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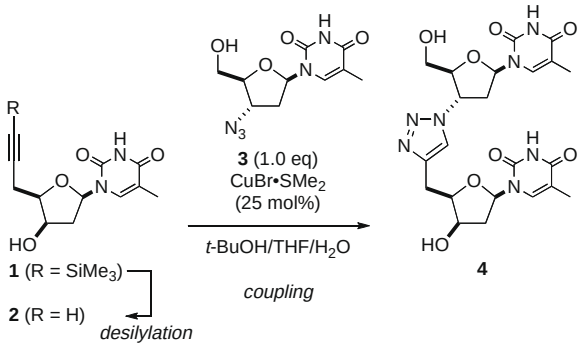
Table 1
One-pot desilylation/coupling reactions for 2-mer ^{TL}DNA

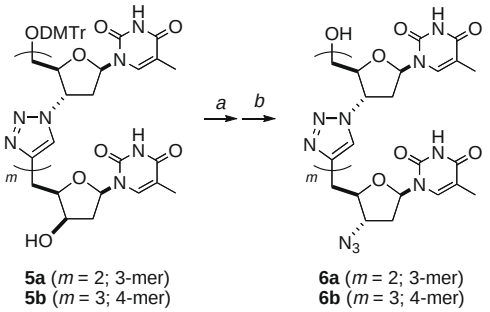


Entry	Conditions		Yield (%)
	Desilylation	Coupling[a]	
1	$AgBF_4$, *t*-BuOH/THF/H_2O[a], rt, 2 h	rt, 12 h	90
2	TBAF, THF, rt, 20 min	rt, 12 h	98
3	Et_3N[b], *t*-BuOH/THF/H_2O[a], 50 °C, 18 h	50 °C, 6 h	96
4	Et_3N[b], *t*-BuOH/THF/H_2O[a], MW[c], 2 h	MW[c], 1 h	97

[a] *t*-BuOH/THF/H_2O = 1:2:3.
[b] 20% v/v.
[c] 80 °C.

TBAF also proceeded, the residual tetrabutylammonium salt was not easily removed and remained contaminated in the oligomer. The coupling between 3′-terminus 4-mer **7** and 5′-terminus 4-mer **6b** also proceeded smoothly in aqueous triethylamine to give 8-mer **8b** in 92% yield.

In summary, we have developed a convergent route for the solution-phase synthesis of oligomers of ^{TL}DNA. The reaction conditions that were optimized for the successive desilylation and click coupling reaction may also be applied to the other cases such as combinatorial chemistry. The simple conditions for reaction and workup allowed the preparation of oligomers in a scale of a few tens of milligrams, which may be applicable to the preparation in a larger scale and thus to the biological and abiological utilities of polythymine ^{TL}DNA in future.[1a,7] In addition, the combination of the solution-phase segmental synthesis of oligomers with solid-phase synthesis may also allow the preparation of a longer polymeric ^{TL}DNA.[8]



Scheme 1. Synthesis of 5′-terminus oligomer of ^{TL}DNA. Reagents and conditions for **6a** (3-mer): (a) (i) MsCl (7.0 equiv), pyridine, rt, 4 h; (ii) NaN_3 (3.0 equiv), DMF, 80 °C, 4 h, 75%; (b) AcOH, rt, 1 h, 97%. Reagents and conditions for **6b** (4-mer): (a) (i) MsCl (7.0 equiv), pyridine, rt, 7 h; (ii) NaN_3 (3.0 equiv), DMF, 80 °C, 6 h, 70%; (b) AcOH, rt, 1 h, 84%.

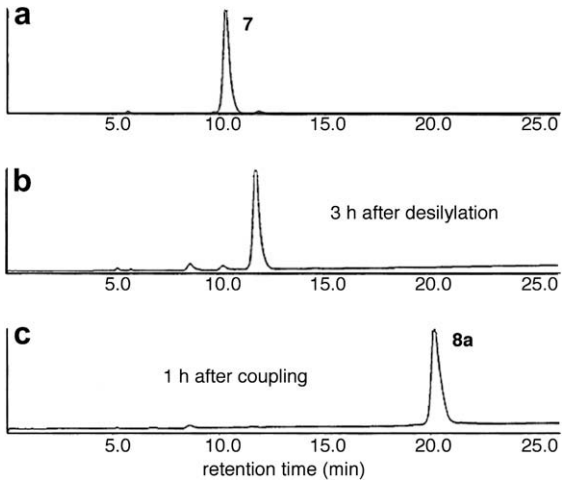
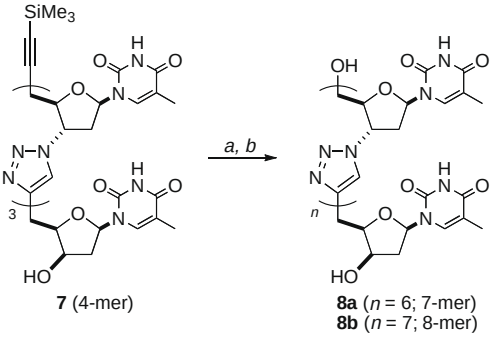


Figure 1. Analysis of one-pot successive reactions for the convergent synthesis of 7-mer **8a** with HPLC (Scheme 2). (a) Before microwave irradiation, 4-mer silylacetylene **7**. (b) Three hours after desilylation of **7** under microwave irradiation in aqueous triethylamine. (c) One hour after the ensuing click coupling reaction under microwave irradiation. HPLC conditions: COSMOSIL triazole-HILIC column, eluent flow gradient from 10% to 30% H_2O (buffered as 10 mM $CH_3CO_2NH_4$, pH 7)/CH_3CN over 30 min at the flow rate of 1 mL/min, monitored at 260 nm.



Scheme 2. Synthesis of 7-mer and 8-mer ^{TL}DNA. Reagents and conditions for **8a** (7-mer): (a) 20% Et_3N in *t*-BuOH/THF/H_2O (1:2:3), MW (80 °C), 3 h. (b) **6a** (1.0 equiv; 3-mer), CuBr·SMe_2 (25 mol %), MW (80 °C), 1 h, 94%. Reagents and conditions for **8b** (8-mer): (a) 20% Et_3N in *t*-BuOH/THF/H_2O (1:2:3), MW (80 °C), 2 h; (b) **6b** (1.0 equiv; 4-mer), CuBr·SMe_2 (25 mol %), MW (80 °C), 1 h, 92%.

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Supplementary data

Supplementary data associated with this paper can be found, in the online version, at doi:10.1016/j.tetlet.2009.04.101.

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6. Taking advantage of the successive procedure, we synthesized 4-mer **5b** by one-pot coupling/desilylation/coupling reactions in good yield, which also shows that the present method can be applied to a sequential 'click–click' chemistry (Ref. 5). See Supplementary data for the experimental details.
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