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Efficient and improved synthesis of triazole-linked DNA (^{TL}DNA) oligomers

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ABSTRACT

A method for the synthesis of triazole-linked DNA oligomers has been revisited to incorporate a reliable protective group and linker for solid-phase synthesis. The new solid-phase synthesis allowed the preparation of oligomers with the efficiency of elongation reaching over 90%.

Solid-phase synthesis has seen great success especially in the preparation of oligomeric compounds such as peptides or oligonucleotides.[1] The success played a key role to ensure their availability and, eventually, to lead the following development of these biopolymers in the field of life science. The applicability of the solid-phase methods considerably affects the development of artificial variants of the biopolymers, as exemplified also by the success of peptide nucleic acid (PNA).[2] Recently, as a new variant of oligonucleotides, we introduced a triazole-linked DNA (^{TL}DNA)[3] and demonstrated its unique function as a lure substrate for an enzyme.[4] For the original synthesis, both solid-phase and convergent solution-phase methods were developed,[3,5] and we found the superiority of solid-phase synthesis particularly in the preparation of long oligonucleotides for bioorganic applications.[4] However, the elongation of ^{TL}DNA oligomers by the solid-phase method has achieved only moderate efficiency (ca. 70–80% per step), which may seriously hamper the future development. In this Letter, we have revised the synthesis method and improved the efficiency of the elongation over 90%.

Two important structural factors, protective groups and linkers, should be examined for successful solid-phase synthesis. In our previous synthesis of ^{TL}DNA oligomers, we adopted trimethylsilyl (TMS) group for the protection of 5′-acetylene moiety in elongating units (**1**) and succinate ester for the linker in 3′-terminus units (**2**; Fig. 1)[6,7] and achieved moderate efficiency for the synthesis of 10-mer oligonucleotide (0.61% yield for 19 steps; 76% yield per step).[3] The analysis of byproducts indicated that the synthesis suffered mainly from the undesired desilylation during copper-catalyzed Huisgen cycloaddition reaction for the elongation. We therefore decided to change the protective group to a more reliable triisopropylsilyl (TIPS) group,[7,8] which consequently required the replacement of the linker unit with adipinate (Fig. 1).[9,10]

We first describe the synthesis of TIPS-protected elongating units. The synthesis route was the same as for the previous TMS-protected elongating unit except that TIPS-protected acetylene was used, and the deoxythymidine-analogue (**3-T**) was prepared from oxetane **5** in two steps in moderate yield (Scheme 1).[11] The synthesis was scalable and allowed the preparation of **3-T** in 40 g

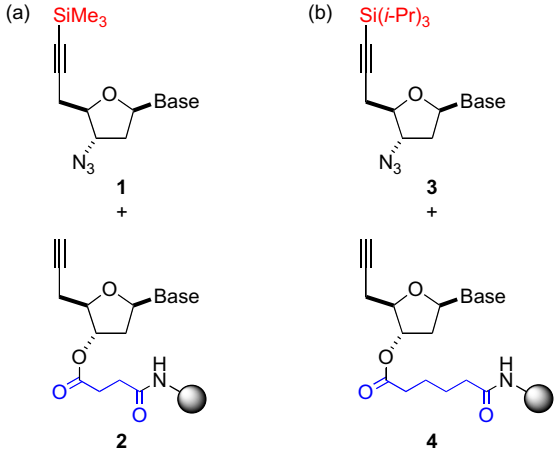

Figure 1. Solid-phase synthesis of ^{TL}DNA showing 3′-termini and elongating units. Colored moieties show the protective group (red) and the linker (blue) that were revised in the present synthesis. (a) Previous synthesis via a TMS/succinate route. (b) Improved synthesis via a TIPS/adipinate route.

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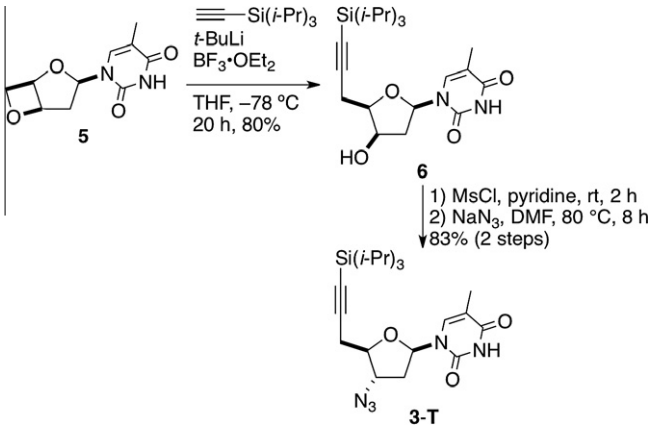


Scheme 1. Synthesis of elongating deoxythymidine-analogue.

through a single sequence of operations. Using **3-T** as a starting material, we also prepared the other three analogues, that is, deoxycytidine-, deoxyadenosine-, and deoxyguanosine-analogues (**3-C**, **3-A,** and **3-G**), through a transglycosylation, similarly, as reported for TMS-protected unit (Fig. 2).[12–14]

The replacement of TMS with TIPS in the protective group requires stronger desilylation reagents such as tetrabutyl ammonium fluoride (TBAF) for the deprotection step,[6] which, consequently, forces us to change the succinate linker.[3,7] After screening a few candidates, we found adipinate as a favorable linker. Thus, we synthesized adipinate with activated ester terminus **8** and loaded it on a solid support (NovaSyn TG amino resin). The loading efficiency was 88% yield and was comparable to that of the succinate linker (Scheme 2).[3] When we desilylated the loaded monomer with TBAF, the deprotected monomer was recovered in 98% yield from **9** after the cleavage from resins (see Supplementary data). The result confirmed that the monomer on the adipinate linker survived the harsh desilylation conditions. Note that the succinate linker could not maintain the 3′-terminus unit during the desilylation with TBAF.[3,7]

Finally, we examined the viability of the new TIPS/adipinate route for the synthesis of a ^{TL}DNA oligomer. As a target for the demonstration, we adopted 12-mer **10** which was active as a primer substrate for the reverse-transcriptase.[4] Thus, repeating the elongation/deprotection steps 11 times (23 steps of synthesis operations), we obtained **10** in 17% yield after hydrolytic cleavage from the resin (Scheme 3). The average yield per step thus was 93%, which was much improved from the previous TMS/succinate route (76% per step).

In summary, we have developed an efficient and improved method for the synthesis of ^{TL}DNA oligomers. Recruitment of reliable moieties for the protection and linkage was successful and enabled us to prepare a primer substrate with the efficiency comparable to that achieved for natural oligonucleotides or

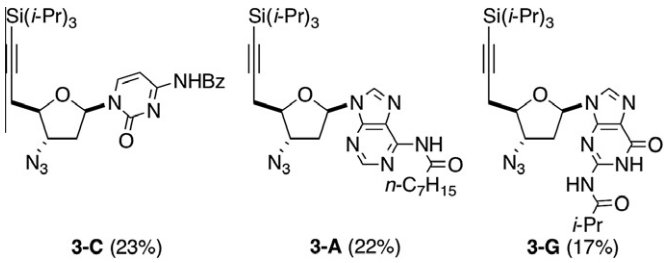
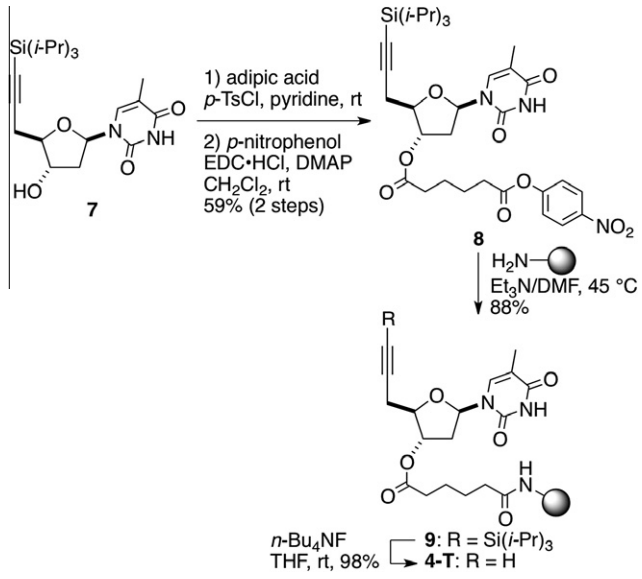
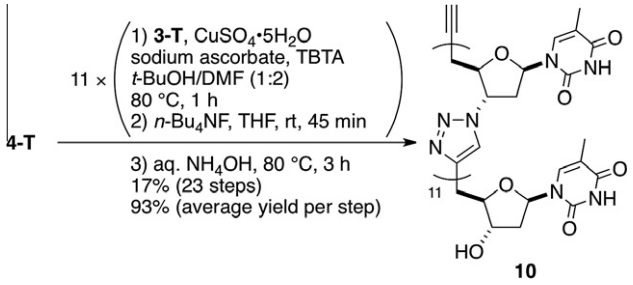


Figure 2. Elongating units with cytosine, adenine, and guanine nucleobases. The numbers in parentheses show the isolated yield of β-analogues through transglycosylation of **3-T**.



Scheme 2. Synthesis of 3′-terminus on a solid support.



Scheme 3. Synthesis of ^{TL}DNA 12-mer via TIPS/adipinate route.

PNA.[2,15] Along with the recent discovery of unique biochemical functions of ^{TL}DNA,[4] the robust synthesis method will help in accelerating the development of new artificial oligonucleotides. The optimized conditions may also be informative for the application of click chemistry to the solid-phase synthesis.[16] We are currently synthesizing functional oligonucleotides with various mixed sequences, which will be reported in near future.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.12.026.

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10. Another possible solution may be an introduction of a capping step to block the unreacted alkyne-terminus for the further reaction. In our preliminary investigation, a positive result with $Co_2(CO)_8$ for the capping reagent has been obtained. However, we did not find the appropriate application for the present synthesis, due to the nearly quantitative result with the TIPS/adipinate method.
11. Synthesis procedures for **3-T** were based on the protocols established for the synthesis of TMS congener (Ref. 3). Physical data of compound **3-T**: IR (powder) 2943, 2174, 2102, 1699, 1465, 1270, 1074, 883 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.07 (s, 21H), 1.92 (d, J = 1.2 Hz, 3H), 2.28 (ddd, J = 7.2, 7.8, 13.6 Hz, 1H), 2.47 (ddd, J = 3.2, 5.8, 13.6 Hz, 1H), 2.72 (dd, J = 5.8, 17.4 Hz, 1H), 2.79 (dd, J = 4.8, 17.4 Hz, 1H), 4.04 (ddd, J = 3.4, 4.8, 5.8 Hz, 1H), 4.27 (ddd, J = 3.2, 3.4, 7.2 Hz, 1H), 6.11 (dd, J = 5.8, 7.8 Hz, 1H), 7.27 (d, J = 1.2 Hz, 1H), 8.68–8.86 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.2 (CH), 12.6 (CH_3), 18.6 (CH_3), 24.9 (CH_2), 37.2 (CH_2), 63.0 (CH), 81.6 (CH), 84.9 (CH), 90.4, 102.1, 111.3, 134.8 (CH), 150.1, 163.5; HRMS (ESI-TOF) calcd for $C_{21}H_{33}N_5O_3SiNa$ $[M+Na]^+$ 454.2250, found 454.2265.
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13. See Ref. 12 for the transglycosylation procedures. Physical data of compound **3-C**: IR (powder) 2943, 2104, 1665, 1484, 1257, 1098, 678 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.07 (s, 21H), 2.33 (ddd, J = 7.4, 8.2, 14.0 Hz, 1H), 2.76–2.82 (m, 1H), 2.77 (dd, J = 4.8, 17.6 Hz, 1H), 2.83 (dd, J = 4.8, 17.6 Hz, 1H), 4.11 (ddd, J = 4.6, 4.8, 4.8 Hz, 1H), 4.23 (ddd, J = 4.6, 4.6, 8.2 Hz, 1H), 6.14 (dd, J = 6.4, 7.4 Hz, 1H), 7.45–7.59 (br s), 7.49 (t, J = 7.6 Hz, 2H), 7.60 (t, J = 7.6 Hz, 1H), 7.90 (d, J = 7.6 Hz, 2H), 8.20 (d, J = 7.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 1.13 (CH), 11.3 (CH_3), 18.7 (CH_3), 24.6 (CH_2), 38.8 (CH_2), 62.4 (CH), 82.4 (CH), 85.4, 86.9, 96.7 (CH), 102.2, 127.7 (CH), 129.2 (CH), 133.1, 133.3 (CH), 143.9 (CH), 154.7, 162.5; HRMS (ESI-TOF) calcd for $C_{27}H_{36}N_6O_3SiNa$ $[M+Na]^+$ 543.2516, found 543.2500. Physical data of **3-A**: IR (oil) 2941, 2096, 1609, 1687, 1462, 1300, 1073, 677, 643 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, J = 6.8 Hz, 3H), 1.08 (s, 21H), 1.28–1.44 (m, 8H), 1.77 (q, J = 7.6 Hz, 2H), 2.58 (ddd, J = 3.8, 6.6, 14.0 Hz, 1H), 2.76 (dd, J = 3.8, 14.6 Hz, 1H), 2.88 (t, J = 8.8 Hz, 2H), 2.89 (dd, J = 8.0, 14.6 Hz, 1H), 3.15 (ddd, J = 6.8, 6.8, 14.0 Hz, 1H), 4.19 (ddd, J = 3.2, 3.8, 8.0 Hz, 1H), 4.52 (ddd, J = 3.2, 3.8, 6.8 Hz, 1H), 6.33 (dd, J = 6.6, 6.8 Hz, 1H), 8.14 (s, 1H), 8.43 (s, 1H), 8.69 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.3 (CH_3), 14.2 (CH), 18.8 (CH_3), 22.8 (CH_2), 25.0 (CH_2), 25.2 (CH_2), 29.2 (CH_2), 29.3 (CH_2), 31.8 (CH_2), 36.8 (CH_2), 38.1 (CH_2), 63.6 (CH), 83.0 (CH), 84.8, 85.1 (CH), 102.5, 122.6, 141.5 (CH), 149.5, 150.8, 152.6 (CH); HRMS (ESI-MS) calcd for $C_{29}H_{46}N_8O_2SiNa$ $[M+Na]^+$ 589.3411, found 589.3391. Physical data of **3-G**: IR (powder) 2942, 2101, 1676, 1608, 1559, 1250, 718 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.07 (s, 21H), 1.29 (d, J = 6.8 Hz, 6H), 2.52 (ddd, J = 3.8, 6.0, 13.8 Hz, 1H), 2.63 (sep, J = 6.8 Hz, 1H), 2.72 (dd, J = 6.8, 17.0 Hz, 1H), 2.75 (ddd, J = 6.6, 7.0, 13.8 Hz, 1H), 2.78 (dd, J = 3.9, 17.0 Hz, 1H), 4.16 (ddd, J = 3.2, 3.8, 7.0 Hz, 1H), 4.41 (ddd, J = 3.2, 3.9, 6.8 Hz, 1H), 6.14 (dd, J = 6.0, 8.0 Hz, 1H), 7.88 (s, 1H), 8.06–8.14 (br s, 1H), 11.9–12.0 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.3 (CH_3), 18.7 (CH_3), 19.1 (CH), 25.4 (CH_2), 36.7 (CH), 37.6 (CH_2), 63.4 (CH), 82.6 (CH), 84.0 (CH), 85.1, 102.2, 121.9, 136.6 (CH), 147.6, 147.9, 153.5, 155.5, 178.4; HRMS (ESI-MS) calcd for $C_{25}H_{38}N_8O_3SiNa$ $[M+Na]^+$ 549.2734, found 549.2711.
14. We also prepared ribonucleoside analogues with the TIPS protective group, which will be reported in due course.
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