HETEROCYCLES, Vol. 87, No. 5, 2013, pp. 1023 - 1028. © 2013 The Japan Institute of Heterocyclic Chemistry
Received, 9th March, 2013, Accepted, 4th April, 2013, Published online, 5th April, 2013
DOI: 10.3987/COM-13-12697

# POST-MODIFICATION OF TRIAZOLE-LINKED ANALOGUES OF DNA FOR POSITIVELY CHARGED VARIANTS

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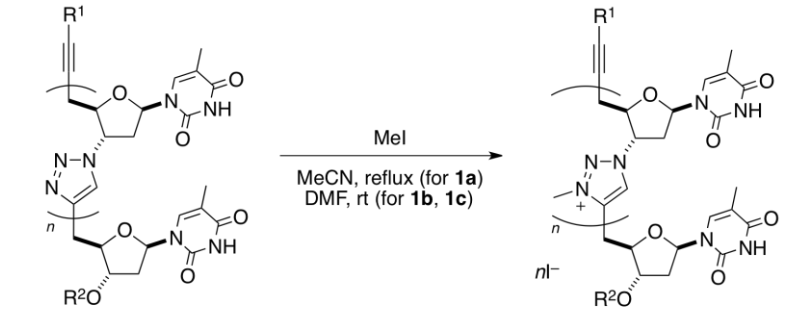
**Abstract** – We report a concise post-modification method to convert an electroneutral triazole-linked DNA ($^{TL}$DNA) to a positively charged triazolium-linked analogue, $^{TL}$DNA+. A one-step methylation of oligothymine $^{TL}$DNA successfully afforded $^{TL}$DNA+ with a dramatic improvement in the solubility. The pentameric oligothymine $^{TL}$DNA+ formed a stable triple helix with natural oligoadenine DNA as well as a mercury-mediated self-duplex.

One of the most common problems accompanied with non-phosphorus, electroneutral DNA analogues is the low solubility of the oligomers. Despite the chemical and biological stability benefitted from the absence of the negatively charged phosphate linkages, the electroneutral strand of oligonucleotides lacks electrostatic supports for the solubility. This solubility issue also arose, when we prepared long oligomers of triazole-linked DNA ($^{TL}$DNA).[1] Specifically, unique functions of the oligothymine $^{TL}$DNA congeners such as electron-transporting materials[2] or lure substrates for reverse-transcriptase[3] prompted us to explore further applications with longer oligomers, but the solubility problem became severer as the oligomers lengthened. We herein report a concise post-modification method for the preparation of positively charged variants, *i.e.*, triazolium-linked analogue of DNA ($^{TL}$DNA+). A one-step methylation of oligothymine $^{TL}$DNA successfully afforded $^{TL}$DNA+ with an improved solubility, and the pentameric oligothymine $^{TL}$DNA+ formed a triple helix with natural oligoadenine DNA in addition to a mercury-mediated self-duplex.

We found that $^{TL}$DNA can be charged positively via a one-step post-modification of oligothymine congeners. Thus, we first examined dimer **1a** with a single triazole unit for the post-modification with methylation reaction.[4] The reaction took place effectively with **1a** and iodomethane at reflux temperature in acetonitrile and, after washing the crude material with chloroform, the corresponding $^{TL}$DNA+ **2a** was obtained in 96% yield as an analytically pure material without chromatographic purification (Scheme 1). We also found that protective groups for 5'-acetylene or 3'-hydroxyl group are unnecessary for this methylation protocol. Tetramer **1b** was thus converted to $^{TL}$DNA+ **2b** with iodomethane in DMF in 71% yield.[5] The same conditions were also applicable to longer **1c** to afford the corresponding pentamer $^{TL}$DNA+ **2c** in 99% yield. The post-modification with methylation was concise enough to be exerted in a large quantity.




**1a** ($n$ = 1, $R^1$ = $SiMe_3$, $R^2$ = $COC_6H_4$-4-$NO_2$) **2a**: 96%
**1b** ($n$ = 3, $R^1$ = H, $R^2$ = H) **2b**: 71%
**1c** ($n$ = 4, $R^1$ = H, $R^2$ = H) **2c**: 99%

**Scheme 1.** Synthesis of oligonucleotides $^{TL}$DNA+.

The positively charged congener, $^{TL}$DNA+, showed an improved and excellent solubility in aqueous media. As a representative example, the solubilities of pentamers were compared. A saturated aqueous solution of the pentamer ($^{TL}$DNA **1c** or $^{TL}$DNA+ **2c**) was prepared in SSPE buffer [10 mM sodium phosphate (pH 7.0), 100 mM sodium chloride and 0.10 mM ethylenediamine tetraacetic acid],[6] and the concentration was determined by measuring the absorbance at 260 nm of an appropriately diluted solution. We determined the saturated concentration in SSPE buffer as 14.5 μM for the neutral $^{TL}$DNA **1c** and 11.8 mM for the positively charged $^{TL}$DNA+ **2c**. The results showed 814-fold improvement in the water solubility via methylation and demonstrated the effectiveness of the post-modification method for the preparation of soluble congeners.

Helix formation of $^{TL}$DNA+ with natural complementary DNA was examined to reveal the affinity improvement of the positively charged variant. In order to obtain reproducible and reliable data for the melting point analysis, we optimized several conditions and found that the sigmoid melting curves were reproducibly obtained with oligoadenine $(dA)_{20}$ as the complementary strand and hexafluorophosphate

($PF_6^-$) as the counter anion of $^{TL}$DNA+ **2c**.[7] During this initial screening, a 2:1 stoichiometry of thymine base (T): adenine base (A) was also indicated for pentamer **2c** and $(dA)_{20}$ (*i.e.*, 8:1 molar ratio of **2c**:$(dA)_{20}$), and we confirmed this optimal ratio by Job plot analysis. As shown in Figure 1a, the Job plot at 0 °C showed the minimal change of $\Delta$%absorbance at 0.7 of the T/(T+A) molar ratio. The result showed the 2:1 stoichiometry of T:A for the optimal complexation and suggested the formation of a triplex with oligothymine **2c** and oligoadenine $(dA)_{20}$.[8] The subsequent analysis of melting curve also confirmed the triplex formation: As can be seen in the representative melting curve for **2c** and $(dA)_{20}$ in Figure 1b, two-phase transitions in the melting curve were detected.[9] The sigmoid curve showed two melting points ($T_m$) at 6 °C and 17 °C, respectively.[10] Considering the Job plot data, we ascribed the first transition to the triplex-to-duplex transition and the second transition to the duplex-to-single strand transition.[11] These $T_m$ values are very high for pentameric oligonucleotides, as the $T_m$ of a natural duplex with $(dT)_5$ can be expected around –33 °C.[12] The neutral $^{TL}$DNA **1c** did not show the melting curve at accessible temperatures, indicating that $T_m$ of neutral **1c** may be below the freezing point of the solvent.[13] We also confirmed the helical arrangement of the three strands with the CD spectrum (Figure 1c).

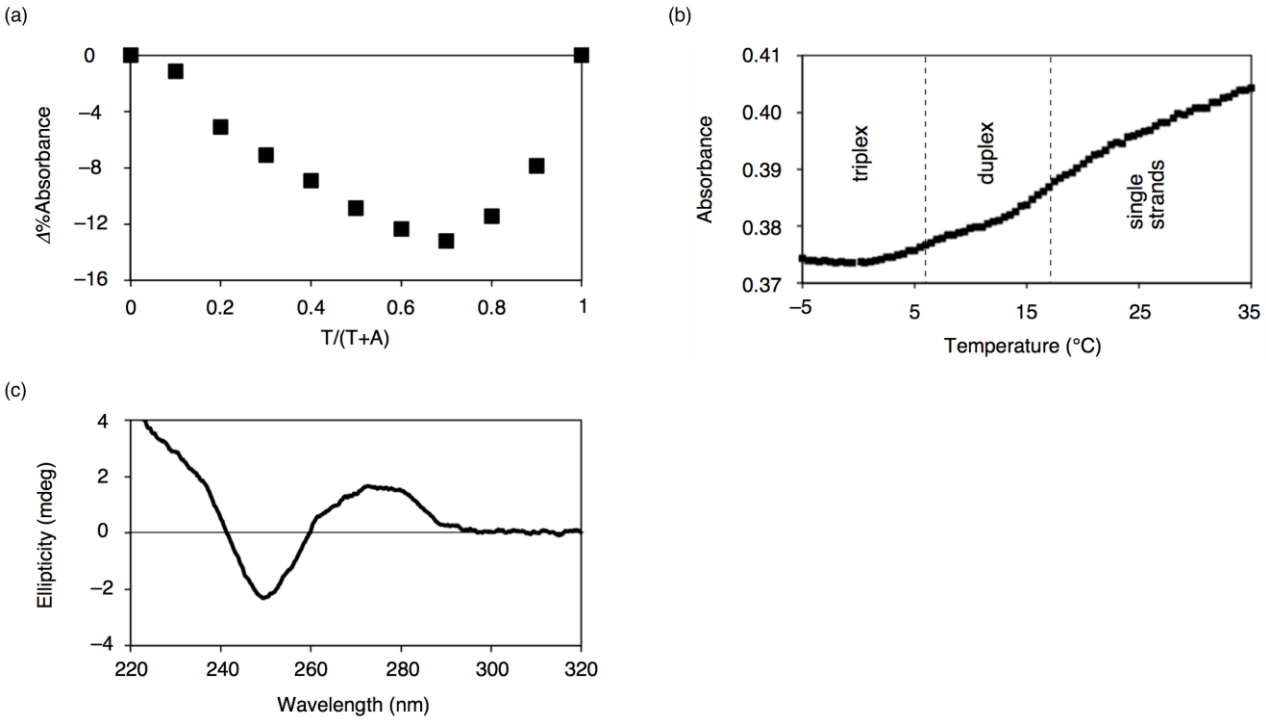



**Figure 1.** Analysis of triplex formation with $^{TL}$DNA+ **2c**. (a) Job plot analysis using %absorbance differences at 260 nm at 0 °C and T/(T+A) for **2c** and $(dA)_{20}$ (See Supporting Information for details). Total base concentration was maintained as 36 μM in sodium phosphate buffer (40 mM, pH 7.0). The minimun point around 0.7 shows the 2:1 stoichiometry of thymine bases and adenine bases for the optimum complex. (b) A thermal denaturation curve for the triplex between $^{TL}$DNA+ **2c** (5.33 μM; 26.7 μM of T) and $(dA)_{20}$ (0.667 μM; 13.3 μM of A) in sodium phosphate buffer (40 mM, pH 7.0) with the heating rate of 0.2 °C/min. (c) A CD spectrum of the triplex of $^{TL}$DNA+ **2c** (5.33 μM; 26.7 μM of T) and $(dA)_{20}$ (0.667 μM; 13.3 μM of A) in sodium phosphate buffer (40 mM, pH 7.0) at 0 °C.

Finally, we examined the mercury-mediated self-duplex formation of $^{TL}$DNA+ **2c**. We previously found that two molecules of the neutral oligothymine $^{TL}$DNA form a duplex with an electron transporting ability on a solid surface through N-Hg-N bond formation with thymine bases.[2] Similarly, upon mixing with $Hg(ClO_4)_2 \bullet nH_2O$ in water/acetonitrile solution, the positively charged oligothymine $^{TL}$DNA+ **2c** formed the mercury-mediated duplex **3** spontaneously. The duplex **3** was detected as the corresponding hydrated species by ESI MS spectrum, and the helical arrangement was confirmed by CD spectrum (Figure 2, S5, S6).[2]

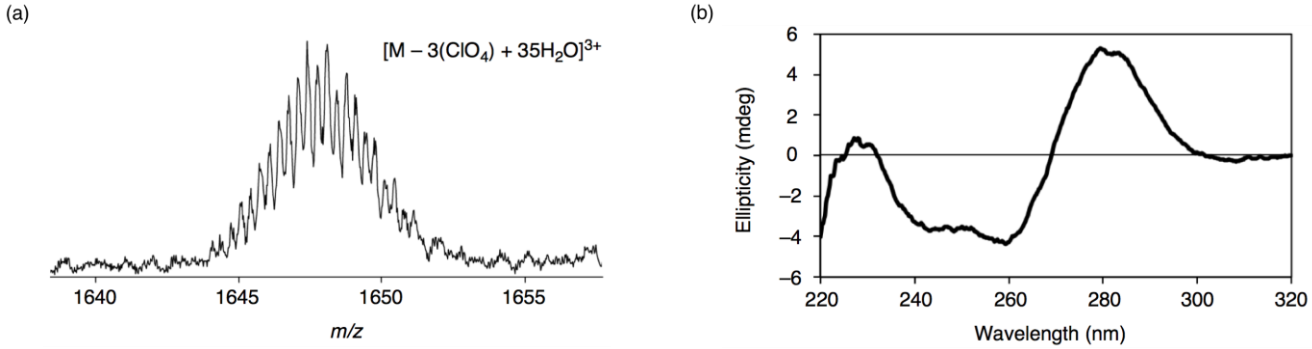



**Figure 2.** (a) A mass spectrum of mercury-mediated duplex **3** obtained by ESI MS measurement. See Figure S3 for the whole range. (b) A CD spectrum of mercury-mediated duplex **3**.

In summary, we developed a post-modification method of $^{TL}$DNA for the positively charged variants, $^{TL}$DNA+. The method is concise enough to be exerted with various triazole derivatives from the click chemistry.[14] The positive charges of the new DNA analogue successfully improved the water solubility and, moreover, binding affinity toward negatively charged natural DNA. The triplex formation with natural DNA may also lead to the development of new functional congeners.[11,15] A mix sequence of $^{TL}$DNA+ and a chimeric combination of $^{TL}$DNA/$^{TL}$DNA+, for instance, through solid-phase and convergent synthesis are another interesting substrates for biological or materials applications in future.

## ACKNOWLEDGEMENTS

We thank Prof. N. Teramae (Tohoku University) for the CD instrument. This work was partly supported by KAKENHI (Nos. 20108015, 23550041 and 24241036).

## REFERENCES AND NOTES

1. (a) H. Isobe, T. Fujino, N. Yamazaki, M. Guillot-Nieckowski, and E. Nakamura, *Org. Lett.*, 2008, **10**, 3729; (b) T. Fujino, N. Yamazaki, and H. Isobe, *Tetrahedron Lett.*, 2009, **50**, 4101; (c) T. Fujino, N. Tsunaka, M. Guillot-Nieckowski, W. Nakanishi, T. Iwamoto, E. Nakamura, and H. Isobe, *Tetrahedron Lett.*, 2010, **51**, 2036; (d) T. Fujino, N. Yamazaki, A. Hasome, K. Endo, and H. Isobe,

*Tetrahedron Lett.*, 2012, **53**, 868.

2. H. Isobe, N. Yamazaki, A. Asano, T. Fujino, W. Nakanishi, and S. Seki, *Chem. Lett.*, 2011, **40**, 318.
3. T. Fujino, K. Yasumoto, N. Yamazaki, A. Hasome, K. Sogawa, and H. Isobe, *Chem. Asian J.*, 2011, **6**, 2956.
4. R. H. Wiley and J. Moffat, *J. Am. Chem. Soc.*, 1955, **77**, 1703.
5. The yield of tetramer **2b** was lower than other cases, because the compound was amphiphilic and therefore dissolved slightly in organic solvents used for precipitation.
6. The saturated solution was prepared as follows: The compound was immersed in SSPE buffer at 50 °C and stirred for 3 h. After gradually cooling the solution to ambient temperature, precipitates were removed by filtration with membrane filter (pore size 200 nm) to afford the saturated solution.
7. We do not understand the detailed mechanism of the condition-dependency of the melting curves at this stage. However, similar effects were observed with cationic guanidium oligonucleotides, which may suggest that the stronger electrostatic interactions with natural strands may affect the helix formation. See for instance: (a) R. O. Dempcy, K. A. Browne, and T. C. Bruice, *Proc. Natl. Acad. Sci. USA*, 1995, **92**, 6097; (b) A. Blaskó, R. O. Dempcy, E. E. Minyat, and T. C. Bruice, *J. Am. Chem. Soc.*, 1996, **118**, 7892.
8. No triplex formation has been detected with electroneutral $^{TL}$DNA under identical conditions. The electronic complementarity between natural DNA and $^{TL}$DNA+ may have facilitated the triplex formation. Similar effects were observed in the neutral thiourea-linked oligonucleotide and cationic *S*-methylthiourea-linked oligonucleotide. See: D. P. Arya and T. C. Bruice, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 691.
9. The hyperchromicity of $^{TL}$DNA upon melting tends to be smaller than that of natural DNA, which has been also observed with the duplex of electroneutral congeners (ref. 1).
10. The $T_m$ values were obtained by calculation of inflection points of the melting curve using a melting program (VWTP-780) from JASCO.
11. (a) N. T. Thuong and C. Hélène, *Angew. Chem., Int. Ed. Engl.*, 1993, **32**, 666; (b) M. D. Frank-Kamenetskii, *Annu. Rev. Biochem.*, 1995, **64**, 65.
12. We calculated the theoretical $T_m$ value of $(dT)_5$•$(dA)_5$ duplex with the following equation: $\Delta H/\{\Delta S + 1.987 \ln(Ct/4)\} - 273.15 + 12.5 \log[Na^+]$ where Ct is the total strand concentration. See: J. SantaLucia, Jr., H. T. Allawi, and P. A. Seneviratne, *Biochemistry*, 1996, **35**, 3555.
13. Electroneutral 10-mer $^{TL}$DNA recorded the $T_m$ value of 61.1 °C (ref. 1).
14. (a) A. H. El-Sagheer and T. Brown, *Chem. Soc. Rev.*, 2010, **39**, 1388; (b) H. C. Kolb and K. B. Sharpless, *Drug Discov. Today*, 2003, **8**, 1128; (c) C. W. Tornøe and M. Meldal, 'Peptides: The Wave of the Future,' ed. by M. Lebl and R. A. Houghten, American Peptide Society and Kluwer

Academic, San Diego, 2001, pp. 263-264.

15. A. S. Boutorine, H. Tokuyama, M. Takasugi, H. Isobe, E. Nakamura, and C. Hélène, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 2462.