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Synthesis and Properties of *D*-Thymidylyl (3'→5')*L*-Thymidine (*D*/*L*-TpT)

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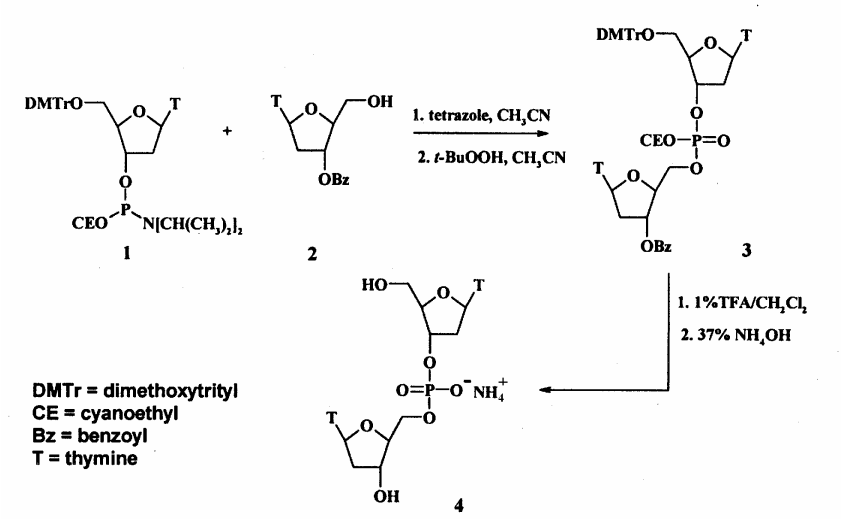
Abstract: The synthesis and properties of *D*-thymidylyl (3'→5')*L*-thymidine (*D*/*L*-TpT) are described.

Keywords: Antisense oligonucleotides, anticancer, synthesis, *D*-thymidylyl (3'→5')*L*-thymidine.

In recent years much effort has been spent to develop oligonucleotide analogs as highly selective pharmaceutical agents to block expression of disease-associated proteins[1-5], and these efforts have resulted in the approval of fomiversen sodium (vitravene^{TM}), the first antisense drug, by FDA in August 1998 for treatment of AIDS patients with cytomegalovirus(CMV)-induced retinitis[6]. However, the great potential of antisense oligonucleotides as antiviral and anticancer agents is compromised by their nuclease susceptibility and low cell membrane permeability. *L*-DNAs have enhanced resistance to nucleases but have been found to hybridize either weakly or not at all with natural RNA and DNA[7-9]. However, preliminary studies suggest that chimeric *D*/*L*-oligomers have adequate duplex-forming capability and excellent enzymatic stability, so they should serve as valuable tools for studying and controlling gene expression in living cells[10-11]. In order to get more insights to the structural features of chimeric *D*/*L*-oligonucleotides, we synthesized *D*-thymidylyl 3'→5')*L*-thymidine (*D*/*L*-TpT) and studied its properties through CD spectrum and 2DNMR spectroscopy. In this communication, we wish to report our preliminary results.

D/*L*-TpT was synthesized using the β-cyanoethylphosphoramidite method[12] without any problems as shown in **Scheme 1**. Condensation of 5'-DMTr-thymidine phosphoramidite **1** with 3'-*O*-benzoyl thymidine **2** in the presence of 1*H*-tetrazole[13] resulted in the formation of an intermediate phosphite triester, which was then oxidized *in situ* to the phosphotriester **3** with *t*-BuOOH[14]. Acidic hydrolysis of the DMTr group from **3** with 1% trifluoacetic acid[15] in CH_2Cl_2 and subsequent removal of the β-cyanoethyl and benzoyl groups by amminolysis (ammonium hydroxide)[16] at ambient temperature for 20 h afforded, after purification (Sephadex LH-20), the desired dinucleoside phosphate **4** in 43% overall yield as a white solid. The identity and homogeneity of **4** was unambiguously ascertained by ^{31}P- and ^{1}H-NMR spectroscopy[17]. We also synthesized d (TpT) as a control by a similar procedure to that described above.

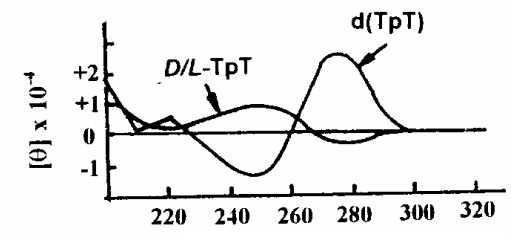
Scheme 1



The CD spectra of *D/L*-TpT and d (TpT) were showed in **Figure 1**. *D/L*-TpT dispalyed flat CD spectrum while d (TpT) showed sharp CD spectrum. *D/L*-TpT had a maximum at 252 nm and a minimum at 278 nm, while d (TpT) had two mixima at 221 nm and 278 nm and a minimum at 248 nm. However, to our surprise, the whole CD curve of *D/L*-TpT demonstrated the characteristics of *L*-nucleoside, which suggested that the 3'-residue(*L*-T) in *D/L*-TpT had a significant effect on the conformation of the dinucleoside phosphate.

The ultraviolet spectral properties of *D/L*-TpT and d (TpT) were measured at pH7 and room temperature. The results indicated that *D/L*-TpT and d (TpT) had the same λ_{max} at 268 nm.

Figure 1



We then investigated the solution conformation of *D/L*-TpT using DQF-COSY, NOESY and TOCSY experiments attempting to understand the effect of introduction of

L-nucleoside on the conformation of the dinucleoside phosphate. We reached those conclusions as below from our studies:

(1) Each base in *D/L*-TpT adopted the *anti* conformer since the NOE effect of H6/H2'is much larger than that of H6/H1'[18-19].

(2) Two sugars in *D/L*-TpT adopted predominantly an *S*-type conformation because its NOESY spectra showed strong H6 to H2'cross-peaks and its COSY spectra also showed strong H1'to H2' and H1'to H2'' cross-peaks (data not shown)[20-21].

Table 1. The selected NOE data of *D/L*-TpT

	D-T ring		*L*-T ring	
H_X-H_Y	1'-6	2'-6	1'-6	2'-6
NOE value	49.565	112.96	57.402	130.92

In summary, we synthesized the chimeric dinucleoside phosphate *D/L*-TpT and investigated its properties comparing with the natural dimer d (TpT). Detailed structural investigations of chimeric *D/L*-oligonucleotides with various base sequences are underway and will be reported in due course.

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References and notes

1. (a) *Prospects for Antisense Nucleic Acid Therapy for Cancer and AIDS*; E.Wickstrom(ed.), Wiley Liss: New York, **1991**.
   (b) *Oligonucleotides as Therapeutic Agents*; Proceedings of the Ciba Foundation Symposium No.209: John Wiley & Sons: Chichester, **1997**.
2 Agrawal, R. P. Iyer, *Curr. Opin. Biotechnol.* **1995**, *6*, 12.
3 C. A. Stein, Y. C. Cheng, *Sciences* **1993**, *261*, 1004.
4 A. De Mesmaeker, R. Haner, P. Martin, H. E. Moser, *Acc. Chem. Res.* **1995**, *28*, 366.
5 S. T. Crooke, C. F. Bennett, *Ann.Rev. Pharmacol. Toxicol*., **1996**, *36*, 107.
6 R. W. Wallace, *Drug Discovery Today*, **1999**, *4*, 4.
7 U. Asseline, J. F. Hau, S. Czernecki, T. L. Diguarher, M. C. Perlat, J. M. Valery, N. T. Thuong, *Nulceic Acids Res.*, **1991**, *19*, 4067.
8 S. Fujimori, Y. Hashimoto, K. Shudo, *J. Am. Chem. Soc*. **1990**, *112*, 7436.
9 A. Garbesi, M. L. Capobianco, F. P. Colonna, L. Todelli, F. Arcamone, G. Manzini, C. Hilbers, J. M. E. Aelen, M. J. J. Blommers, *Nucleic Acids Res.* **1993,** *21*, 4159.
10 M. Damha, P. A. Giannaris, P. Marfey, *Biochemistry* **1994**, *33*, 7877.
11 M. J. J. Blommers, L. Tandelli, A. Garbesi, *Biochemistry* **1994**, *33*, 886.
12 N. D. Sinha, J. Biernat, H. Koster, *Tetrahedron Lett*. **1983**, *24*, 5843.
13 N. D. Sinha, J. Biemat, J. McManus, H. Koster, *Nucl.Acids Res*. **1984**, *12*, 4539.
14 Y. Hayakawa, M. Uchiyama, R. Noyori, *Tetrahedron Lett*. **1980**, *27*, 4191.
15 M. Fujji, K. Ozaki, M. Sekine, T. Hata, *Tetrahedron* **1987**, *43*, 3395.
16 M. D. Matteucci, M. H. Caruthers, *J.Am.Chem.Soc.* **1981**, *103*, 3185.
17 Data for **4**: R_f=0.29 (*i*-PrOH/NH_4OH/H_2O=26:6:6); **^{31}P-NMR** (D_2O) δ (ppm): -0.814; **^{1}H-NMR**(D_2O) δ(ppm): 7.74 (s, 1H, H_B-6), 7.67 (s, 1H, H_A-6), 6.37 (t, J=6.78 and 6.84Hz, H_B-

1'), 6.30 (t, 1H, J=6.84 and 6.84Hz, H_A-1'), 4.82 (m, 1H, H_A-3'), 4.60 (m, 1H, H_B-3'), 4.22 (m, 1H, H_A-4'), 4.21 (m, 1H, H_B-4'), 4.18 (m, 1H, H_B-5'), 4.12 (m, 1H, H_B-5''), 2.56 (m, 1H, H_A- 2''), 2.41 (m, 1H, H_A-2'), 2.44 (m, 2H, H_B-2', H_B-2''), 1.94 (s, 3H, CH_{3A}-5), 1.93 (s, 3H, CH_{3B}- 5). **HRMS** (FAB): calcd for $C_{26}H_{42}N_5O_{12}P$ 647.556, found 647.545.

18 K. Wuthrich, "*NMR of Proteins and Nucleic Acids*", John Wiley & Sons: New York, **1986**, pp203.

19 G. M. Clore, A. M. Gronenborn, *FEBS Lett*. **1985**, *179*, 187.

20 K. V. R. Chary, S. Modi, R. V. Hosur, G. Govil, *Biochemistry* **1989**, *28*, 5240.

21 J. Micklefield, K. J. Fettes, *Tetrahedron* **1998**, *54*, 2129.

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