

## Modified Triplexes from Oligonucleotides Bridged by Two Cholane-3,24-diol ( $3\alpha,5\beta$ ) Units<sup>\*</sup>

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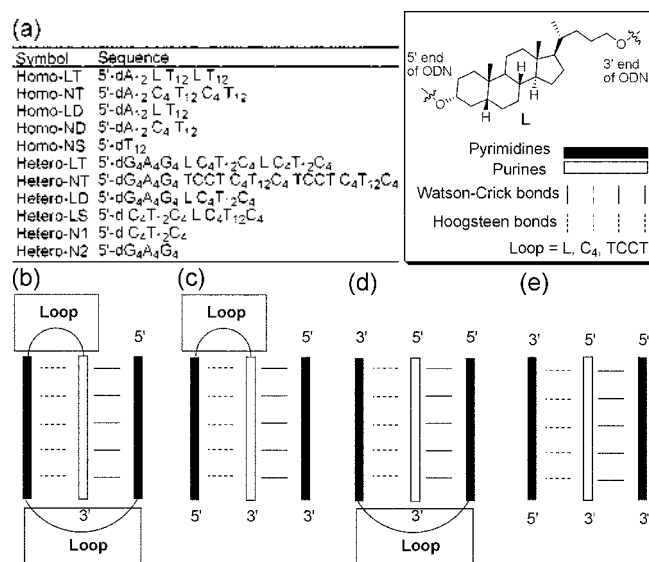
**Key Words :** Triplex. Hairpin oligonucleotide. Phosphoramidite

Since the first discovery of triple helix (triplex),<sup>1</sup> it has been suggested that a biologically important three-stranded complex could be constructed from single-stranded RNA and double-stranded DNA. In general, a triplex is formed when a third strand binds in the major groove of a B-form double helix (duplex). The duplex must be composed of a homopurine-homopyrimidine sequence, which represents up to 1% of eukaryotic genomes.<sup>2</sup> Although direct evidence of the participation of triplexes in biological processes has yet to be obtained, a growing body of data suggests that triplexes are involved in the regulation of DNA replication, transcription ("antigene therapy"), recombination, and development.<sup>3</sup> In addition, triplexes have been demonstrated to be stable under physiological conditions and to inhibit various enzymes, including RNA polymerase,<sup>4</sup> DNase, and RNase.<sup>5</sup>

Recently, we synthesized a novel phosphoramidite reagent, 24-*O*-(4,4'-dimethoxytrityl)cholane-3,24-diol-3-(2-cyano-

ethyl-*N,N*-diisopropylphosphoramidite ( $3\alpha,5\beta$ ) (L), and used it to develop hairpin oligonucleotides.<sup>6</sup> We became interested in extending this research to the development of stable triple-stranded nucleic acids incorporating two hairpin moieties (Figure 1);<sup>7</sup> such stable triplexes may be useful tools for studying the biological roles and genetic applications of naturally occurring triplexes.

We synthesized the requisite modified oligodeoxynucleotides (ODNs) with high coupling efficiencies using an automated DNA synthesizer (Figure 1a) and confirmed their successful syntheses through MALDI-TOF mass spectral analysis. The ODNs **Homo-LT**, **Homo-NT** and **Hetero-LT** are palindromers containing two hairpin moieties; we expected that these modified ODNs might form triplexes that feature intramolecular hydrogen bonding patterns. Table 1 summarizes the melting temperature data of the ODN systems. In general, the intrastrand triplexes were more stable than the interstrand triplexes and replacement of C<sub>4</sub> hairpin residues by the L group provided extra stability to the triplexes. **Homo-LT** and **Homo-NT** exhibit single melting temperatures (entries 1 and 2) that reflect the fact that the melting of the Hoogsteen strands occurs at the same temperature as does that of the Watson-Crick strands (*i.e.*, the strands melt simultaneously). In addition, we note that



**Figure 1.** Possible triplex structures of the designed ODNs: (a) Sequences of the synthetic ODNs; (b) **Homo-LT**, **Homo-NT**, **Hetero-LT**; (c) **Homo-LD** or **Homo-ND/Homo NS**, **Hetero-LD/Hetero N1**; (d) **Hetero-LS/Hetero-N2**; (e) 2 (**Hetero-N1**)/**Hetero-N2**.

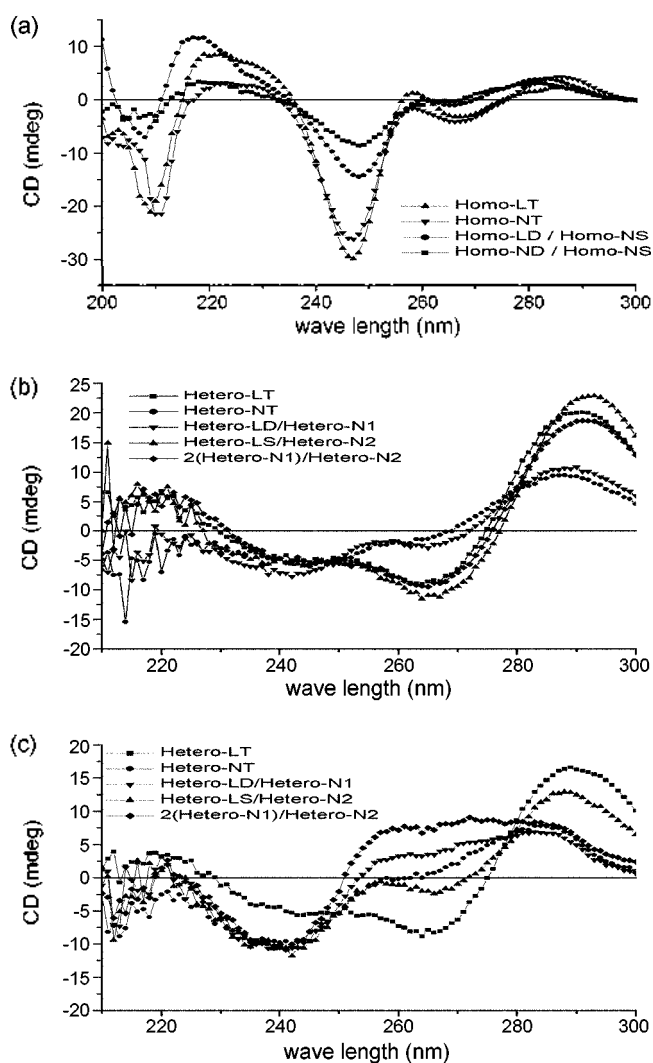
**Table 1.** Melting Temperatures of Synthetic ODNs<sup>a</sup>

Entry	Name	$T_m$ (°C)	$T_m$ (°C)	$T_m$ (°C)
		at pH 5.0	at pH 6.0	at pH 7.2
1	<b>Homo-LT</b>	—	—	76 (71 <sup>b</sup> )
2	<b>Homo-NT</b>	—	—	69 (62 <sup>b</sup> )
3	<b>Homo-LD / Hetero N1</b>	—	—	17, 75 (17 <sup>b</sup> )
4	<b>Hetero-LS/Hetero-N2</b>	—	—	19, 70 (19 <sup>b</sup> )
5	<b>Hetero-LT</b> <sup>c</sup>	65, 82	73	72
6	<b>Hetero-LD</b> <sup>c</sup>	52	70	71
7	<b>Hetero-LD/Hetero-N1</b> <sup>c</sup>	46	44, 69	16, 71
8	<b>Hetero-LS/Hetero-N2</b> <sup>c</sup>	77	44, 66	34, 53
9	<b>2(Hetero N1)/Hetero N2</b>	56 (46 <sup>c</sup> )	36, 63 (31 <sup>c</sup> )	63 (35 <sup>c</sup> )

<sup>a</sup>Values of  $T_m$  were determined by measuring changes in absorbance at 260 nm (cuvette, 1-cm path length) as a function of temperature in 10 mM buffer solution [either sodium acetate (pH 5.0), Tris-acetate (pH 6.0), or Tris-HCl (pH 7.2) buffer solution] containing 10 mM of NaCl and 20 mM of MgCl<sub>2</sub>. The total ODN concentration was 3  $\mu$ M. The temperature was raised at a rate of 1.0 °C/min. <sup>b</sup>The data were obtained by monitoring at 284 nm. <sup>c</sup>The buffer solution was prepared without NaCl or MgCl<sub>2</sub> and the solution was monitored at 260 nm.

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**Figure 2.** CD spectra of ODNs recorded at 260 nm in buffer solutions at (a) pH 7.2 (in 10 mM NaCl and 20 mM MgCl<sub>2</sub>), (b) pH 5 [same buffer as (a)], and (c) pH 7.2 (in the absence of NaCl and MgCl<sub>2</sub>). The conditions are described in Table 1. All spectra were recorded at 10 °C.

**Homo-LT** has higher values of  $T_m$  (measured at both 260 and 284 nm) than does **Homo-NT**. In the cases of the hetero-sequences (entries 5 to 9), we observe that the thermal stability of the third strand in the triplex increases as the solution becomes more acidic, consistent with the fact that protonated C more effectively binds to major groove of C-G base pairing to form triplex.<sup>7b</sup> The strands of **Hetero-LT** melt simultaneously at both pH 6 and 7, but in a stepwise manner at pH 5 (entry 5). By comparing this phenomenon with the results presented in entry 6, we believe that the first melting curve of **Hetero-LT** reflects the melting of the duplex and

the second reflects melting of the third strand.<sup>8</sup>

Figure 2 presents the superimposed CD spectra of the triplexes. The appearance of the strong negative CD band (210–220 nm) in Figure 2a indicates the triplex formation. The CD spectra of the ODNs having hetero-sequences (Figures 2b–c) are consistent with those obtained in preceding studies.<sup>9</sup> From the electronic effect provided by protonated C, the typical spectra of a nucleic acid duplex in the B conformation shifted slightly toward longer wavelength. In addition, **Hetero-LT** formed its triplex under neutral as well as acidic pH conditions.

In conclusion, we have designed and synthesized intra-strand triplexes using a novel phosphoramidite monomer prepared from lithocholic acid as a structural scaffold upon which we attached the hairpin residues. We have confirmed the nature of the secondary structures of these ODNs by determining their values of  $T_m$  and by performing semi-empirical analyses of CD spectroscopic data. These analyses reveal that the modified ODNs form highly stable triplexes through intramolecular hydrogen bonding between their complementary sequences of ODNs. The L moiety provides greater stability to the hairpin structure than exists in natural DNA hairpin structures.

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