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# NOVEL OLIGODEOXYRIBONUCLEOTIDES INCORPORATING L-RELATED ISODEOXYNUCLEOSIDES: SOLID PHASE SYNTHESIS, ENZYMOLOGY, AND CD STUDIES

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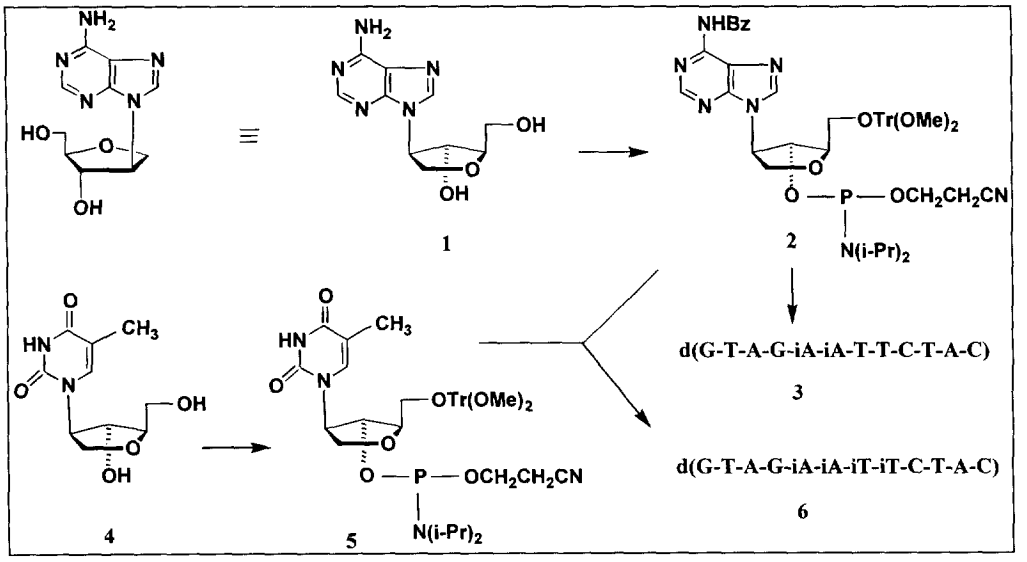
**Abstract:** The synthesis of self-complementary oligodeoxyribonucleotides incorporating the L-related isodeoxynucleosides, isodA, (**1**) and/or isodT (**4**) is described. These novel oligomers exhibit high resistance towards exonucleases. Temperature- and concentration-dependent CD spectra and thermodynamic data of the oligomers show that isodA-isodT base pairs participate in duplex stabilization. © 1997 Elsevier Science Ltd.

The building blocks of natural DNA contain three chiral C-atoms, one at the anomeric center and two in the sugar-phosphate backbone. There are several reports on ribosyl-modified oligodeoxynucleotides with configurational changes at the 3′-position and the 4′-position.[1-6] These changes lead to DNA structures with interesting structural characteristics and biological properties. Hitherto unknown, however, are oligonucleotides in which the nucleobase at the natural C-1′-position is transposed to the isomeric 2′-position, which is also the equivalent of transposition of the endocyclic oxygen to the 3′-position. Recently, we have reported on the synthesis of isomeric deoxy- and dideoxynucleosides including (*S,S*)-isodideoxyadenosine [(*S,S*)-isoddA].[7,8] This dideoxynucleoside was resistant to chemical and enzymatic degradation and had potent antiviral activity against HIV-1, HIV-2, and HIV-1 clinical isolates.[9] (*S,S*)-IsoddA 5′-triphosphate was a powerful inhibitor of the viral DNA polymerase, HIV reverse transcriptase ($K_i$ 16 nM).[9] In order to gain a better understanding of the special characteristics that would be acquired by oligodeoxynucleotides incorporating isomeric nucleosides, including those isonucleosides that exhibit antiviral activity through viral DNA chain termination, we have investigated the synthesis, enzymology, and properties of such conceptually new, self-complementary oligodeoxynucleotides. This is the focus of the communication.

The isomeric deoxynucleoside precursors (**1** and **4**) were synthesized by modification of methods developed in our laboratory.[8-12] Nucleosides **1** and **4** were converted into the β-cyanoethyl phosphoramidite building blocks **2** and **5** for oligonucleotide synthesis. Compound **1** was N-benzoylated first (transient-protection protocol[13]), and then the 5′-hydroxyls of **1**(NHBz) and **4** were protected with the 4,4′-dimethoxytriphenylmethyl group. Subsequent treatment with chloro(diisopropylamino)-β-cyanoethoxyphosphine furnished the phosphoramidites **2** and **5** as diastereoisomeric mixtures. These new compounds were characterized by elemental analysis and $^{1}H$, $^{13}C$, and $^{31}P$ NMR spectral data.[14,15] Automated solid-phase synthesis[16] (Applied Biosystems) with **2** and **5** furnished the target oligonucleotides. Incorporation of the isomeric DNA building blocks required much longer coupling times (10x) than the corresponding 2′-deoxyribophosphoramidites. Coupling yields based on the liberation of the 4,4′-dimethoxytrityl cation at 498 nm reached 70% for **2** and 80% for **5**.




Enzymatic hydrolysis of the isolated, purified oligonucleotides **3** and **6** by exonucleases was studied (Figs. 1 and 2) and showed significant resistance of the phosphodiester bonds towards exonucleases, with resistance increasing with the number of isomeric nucleosides within the structure of the oligonucleotide. The enzyme kinetics follow a discontinuous two-step mechanism. In the first step, the unmodified nucleotides are hydrolyzed with close to normal rates [e.g., as for d(GTAGAATTCTAC) **7**], whereas in the second step, the inner part is hydrolyzed by the enzymes at a much lower rate (Figs. 1 and 2).

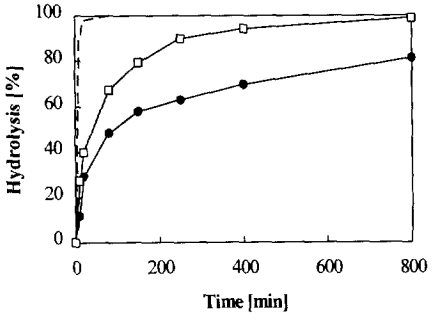



Figure 1

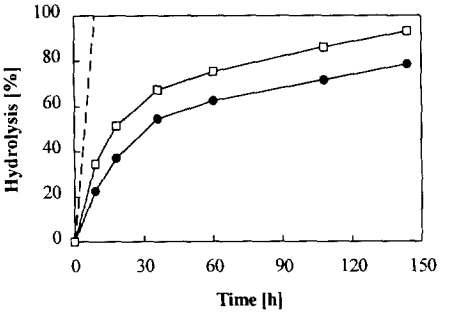



Figure 2

**Figure 1 and 2.** Time course of phosphodiester hydrolysis of **3** (□) and **6** (•) in 0.1 M Tris-HCl buffer (pH 8.3) at 37 °C; Fig. 1: snake-venom phosphodiesterase (3′-5′-exonuclease), Fig. 2: calf-spleen phosphodiesterase (5′-3′-exonuclease), both followed by alkaline phosphatase treatment. Hydrolysis of the unmodified oligonucleotide **7** is also shown in both figures (- - - - -).

The structural stability of the modified oligonucleotides **3** and **6** was studied using temperature-dependent CD spectroscopy. For this purpose, both π-π* transitions ($B_{1u}$, $B_{2u}$) of **3** and **6** were measured as a function of temperature between 10–70 °C. Oligonucleotides **3** and **6** showed cooperative melting-profiles (Fig. 3). Furthermore, both π-π* transitions exhibited almost the same $T_m$ value.

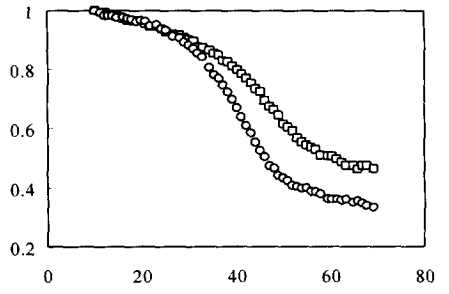



Figure 3

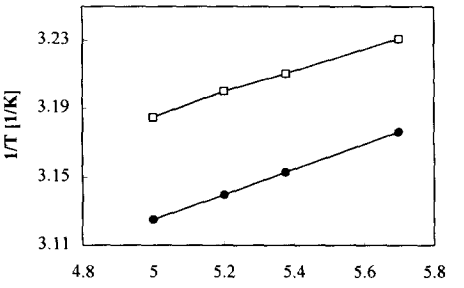



Figure 4

**Figures 3 and 4**. Normalized CD-melting profiles of **3** (□) and **6** (o) in 1 M NaCl containing 100 mM $MgCl_2$, 60 mM Na-cacodylate (pH 7.0); single strand concentration, 5 μM (Fig. 3); -log c vs. $T_m^{-1}$ plot, **3** (□) and **6** (•) (Fig. 4).

In order to establish whether a duplex or hairpin was formed, the thermal denaturation curves of **3** and **6** as a function of oligomer concentration were determined (Fig. 4). Reduction of the oligomer concentration from 10 μmol to 2 μmol led to a decrease in the $T_m$ value by 4–5 °C which is suggestive of duplex melting. We also determined the thermodynamic parameters for **3** and **6** ($T_m^{-1}$ vs -log c).[17-19] If the inner modified tetramer [d(iAiAiTiT)] within **6** does not take part in base pairing, a ΔH value of about -70 kcal/mol would be expected; the ΔH value would increase to approximately -95 kcal/mol, if two stable Watson–Crick H-bonds are formed. As can be seen from Table 1, incorporation of two isodA-building blocks **1** within oligomer **3** does not disturb duplex formation. Even replacement of the innermost part by both **1** and **4** (oligomer **6**) appears to maintain base-pairing interaction within the duplex structure. However, the enthalpic contribution of each isodA-isodT base pair is slightly lower than that of the normal dA-dT base pair (Table 1).

In summary, the L-related isodeoxynucleosides **1** and **4** were successfully incorporated within self-complementary oligonucleotides. These conceptually novel oligomers exhibit high resistance towards exonucleases. Temperature-dependent CD spectral data show cooperative melting profiles. Enthalpic data provide support for the participation of the isodA-isodT base pairs in duplex stabilization. Syntheses of further novel isooligonucleotides are under investigation.

**Table 1.** $T_m$-Values and Thermodynamic Data for the Oligomers **3**, **6**, and **7**.[a]

| Oligomer | $h_{enzyme}$[b] [%] | $T_m$ [°C] | ΔH [kcal/mol] | ΔS [cal/mol K] | ΔG° [kcal/mol] |
|---|---|---|---|---|---|
| d(GTAGiAiATTCTAC) (**3**) | 18 | 45 | -97.7 | -307 | -6.2 |
| d(GTAGiAiAiTiTCTAC) (**6**) | 12 | 39 | -86.2 | -276 | -3.9 |
| d(GTAGAATTCTAC) (**7**) | 31 | 48 | -90.1 | -280 | -6.6 |

a. Measured in 1 M NaCl containing 100 mM $MgCl_2$ and 60 mM Na-cacodylate (pH 7.0); single strand concentration, 5 μM. b. Enzymatic hypochromicity (snake-venom phosphodiesterase).

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14. Data for compound **2**: $^1$HNMR ($CDCl_3$) δ 0.96–1.12 (m, *14H*, $(CH_3)_2CH$), 2.28 (m, *2H*, $CH_2CH_2CN$), 2.53 (m, *2H*, $CH_2CH_2CN$), 3.44–3.57 (m, *2H*, $CH_2OH$), 3.76 (s, *3H*, $OCH_3$), 3.77 (s, *3H*, $OCH_3$), 4.10–4.18 (m, *1H*, H-2′), 4.38 (m, *2H*, H-5′), 4.80 (m, *1H*, H-3′), 5.24 (m, *1H*, H-4′), 6.77–6.83 (m, *4H*, arom. H), 7.18–7.32 (m, *7H*, arom. H), 7.38–7.44 (m, *2H*, arom. H), 7.53 (t, *2H*, arom. H), 7.60 (t, *1H*, arom. H), 8.03 (d, *2H*, arom. H), 8.23 (s, *1H*, H-2), 8.80 (s, *1H*, H-8), 9.02 (s (br), *1H*, N*H*-6); $^{31}$PNMR ($CDCl_3$) δ 151.2, 151.4.
15. Data for compound **5**: $^1$HNMR ($CDCl_3$) δ 1.04–1.25 (m, 14H, $(CH_3)_2CH$), 1.54 (s, *3H*, $CH_3$), 2.20 (m, *2H*, $CH_2CH_2CN$), 2.56 (m, *2H*, $CH_2CH_2CN$), 3.41–3.51 (m, *2H*, $CH_2OH$), 3.71 (s, *3H*, $OCH_3$), 3.72 (s, *3H*, $OCH_3$), 3.82–3.88 (m, *1H*, H-2′), 3.98–4.15 (m, *2H*, H-5′), 4.52 (m, *1H*, H-3′), 5.09 (m, *1H*, H-4′), 6.75 (m, *4H*, arom. H), 7.14–7.26 (m, *7H*, arom. H), 7.34 (m, *3H*, H-4/arom. H), 8.30 (s, *1H*, N*H*-1); $^{31}$PNMR ($CDCl_3$) 151.6, 151.9.
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