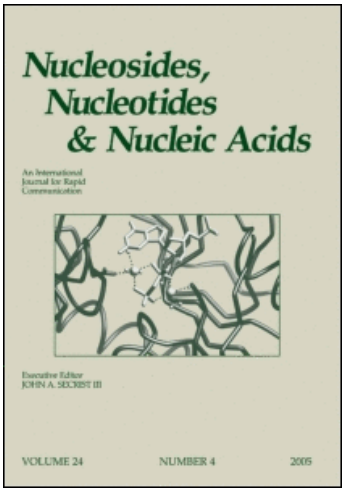

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Control of DNA Conformation Using 3'-S-Phosphorothiolate-Modified Linkages

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CONTROL OF DNA CONFORMATION USING 3′-S-PHOSPHOROTHIOLATE-MODIFIED LINKAGES

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▫ *An in-depth study into the incorporation of multiple 3′-S-phosphorothiolate modifications into oligodeoxynucleotides (ODNs) and their subsequent effect on ODN/DNA and ODN/RNA duplex stability. 3′-S-Phosphorothiolate linkages increase the stability of ODN/RNA duplexes and decrease the stability of ODN/DNA duplexes.*

INTRODUCTION

Synthesis of oligodeoxynucleotides with an enhanced binding affinity for RNA has become particularly desirable over the past years in respect to the development of antisense therapeutics. ^{1}H NMR studies by Beevers et al.[1–3] have shown that if the natural phosphodiester linkage in a thymidine dimer is replaced by a 3′-S-phosphorothiolate linkage (where the 3′-oxygen is replaced by sulfur, see Figure 1), there is a shift in the conformational equilibrium of the sugar directly attached to the sulfur atom from the south to the north pucker. This is attributed to the less electronegative sulfur atom reducing the *gauche* effects within the deoxyribose ring, which would otherwise stabilize the south conformation. It has also been demonstrated that this conformational shift also occurs, but to a lesser extent, in the (n + 1) sugar, thereby resulting in the dimer existing in an RNA- rather than a DNA-type conformation [1,2]

Incorporation of the 3′-S-phosphorothiolate linkage into the dodecamer 5′d($C_1C_2T_3A_4A_5A_6T_7T_{8(S)}T_9G_{10}C_{11}C_{12}$) produced an almost identical result.[1–3] when found in a duplex with its RNA complement. The sugar directly attached to the sulfur atom (T_8) and the (n + 1) sugar (T_9) were both found to adopt a

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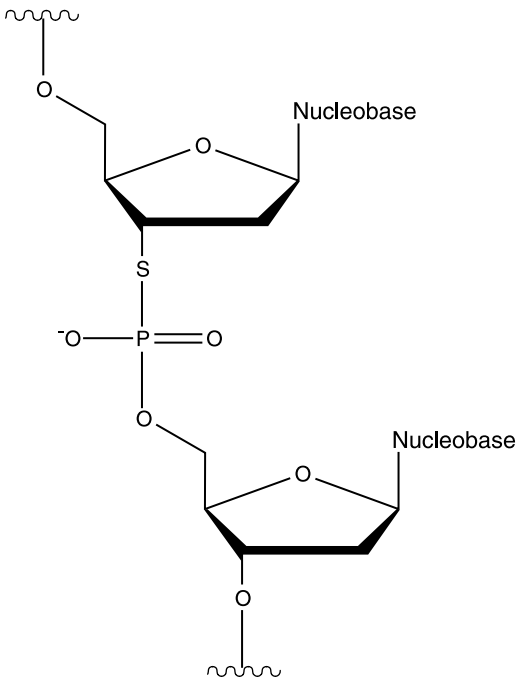

FIGURE 1 3′-S-phosphorothiolate linkage.

predominantly north pucker, whereas identical sugars in the unmodified dodecamer display the more typical south conformation.[1] An enhancement in the stability of the duplex formed between the modified dodecamer and its RNA complement (ΔT_m = +2.5°C) compared to the equivalent unmodified duplex was also observed. Further studies indicated that altering the position of a single modification has minimal impact on the T_m of duplexes formed with complementary RNA. However, incorporating two adjacent modifications into the oligodeoxynucleotide increases the T_m of these systems by + 3°C and incorporating two alternating phosphorothiolate linkages enhances stability even further (ΔT_m = +4°C).[3] Work by Peterson et al.[4] on LNA modifications also shows a thermodynamic advantage to alternating modifications as well as a saturation level in terms of the extra stability additional modified units can bring. However, investigations into this feature using the dodecamer 5′d($C_1C_2T_3A_4A_5A_6T_7T_{8(S)}T_9G_{10}C_{11}C_{12}$) are limited by the fact that there are only three consecutive T residues in the sequence. In addition, this sequence is known to adopt an unusual structure with a kink at the central AT step. For these reasons the sequence 5′d($GCGT_{10}GCG$) was chosen in this present study to investigate the effect of multiple modifications.

RESULTS AND DISCUSSION

Previously, we developed a general, automated procedure for the synthesis of oligodeoxynucleotides containing 3′-S-phosphorothiolate linkages using

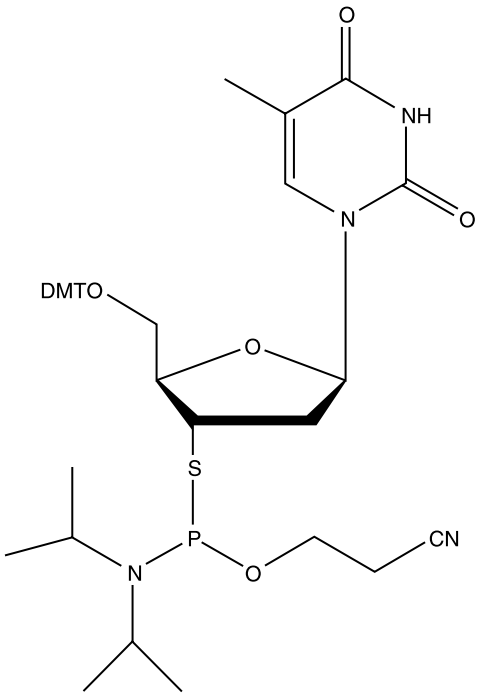

FIGURE 2

3′-S-(2-cyanoethyl-N, N-diisopropylphosphorothioamidite)-3′-deoxy-5′-O-(4, 4′-dimethoxytrityl)thymidine (Figure 2).[5]

Phosphoramidite chemistry was used to synthesize all oligodeoxynucleotides shown in Table 1 on a 1-μmole scale. Slight alterations were made to the thioamidite coupling step in the solid phase synthesis; the coupling time was extended to 15 min, the activator used was 1.0 M 5-ethyl-^{1}H-thiotetrazole, and the concentration of the thioamidite solution was 0.15 M. T_m values of duplexes formed between equimolar mixtures (3 μM strand concentrations) of each oligodeoxynucleotide and its complementary DNA 5′d(CGCA$_{10}$CGC) and RNA

TABLE 1 Duplex Melting Temperatures of 3′-S-Phosphorothiolate–Modified Oligonucleotides with Complementary DNA and RNA

ODN	Sequence 5′ → 3′	T_m (°C) *vs.* RNA	T_m (°C) *vs.* DNA
ODN 1	GCGTTTTTTTTTTGCG	39.7	43.3
ODN 2	GCGTTTTT$_s$TTTTTGCG	40.7	42.6
ODN 3	GCGTTT$_s$TT$_s$TTTTTGCG	42.5	41.9
ODN 4	GCGTTT$_s$TT$_s$TT$_s$TTTGCG	43.6	40
ODN 5	GCGT$_s$TT$_s$TT$_s$TT$_s$TTTGCG	45.1	39
ODN 6	GCGT$_s$TT$_s$TT$_s$TT$_s$TT$_s$TGCG	46.5	38.6
ODN 7	GCGTTTT$_s$T$_s$TTTTTGCG	41.8	41.7

T_m = melting temperature, $T_{(s)}$ = 3′-thiothymidine (phosphorothiolate linkage).

5′r(CGCA$_{10}$CGC) strands were determined (Table 1). All mixtures were prepared in a phosphate buffer (10 mM, pH 7.0) with a duplex concentration of 3 μM.

As expected, due to conformational compatibility, the duplex formed between the unmodified oligodeoxynucleotide, ODN1, and its DNA complement is more stable than the hybrid formed with its RNA complement (ΔT_m = +3.6°C). As the number of alternating 3′-S-phosphorothiolate modifications in the oligodeoxynucleotide is increased (ODN 2 to ODN 6), the T_m values, and hence the stability of duplexes formed with the DNA complement, were observed to decrease. Conversely, the T_m values and stability of the duplexes formed with complementary RNA were seen to increase. This is consistent with an increasing number of 3′-S-phosphorothiolate linkages forcing progressively more sugars to adopt a north pucker, resulting in the oligodeoxynucleotide having a more RNA-like conformation. When three or more alternating modifications are incorporated into the oligodeoxynucleotide (ODNs 4 to 6) duplexes formed with the RNA complement exhibit a greater stability than that of the unmodified DNA duplex. ODN 3 forms a marginally more stable duplex with complementary RNA than ODN 7. This result supports the observation that the 3′-S-modification not only alters the conformation of the sugar to which it is directly attached, but also steers the sugar of the (n + 1) nucleotide from a south to a north pucker. This is because in ODN 3 there are two (n + 1) sugars that are perturbed, but in ODN 7 there is only one, and thus the overall conformation of ODN 7 is less RNA-like than ODN 3.

CONCLUSIONS

As the number of 3′-S-phosphorothiolate modifications in the sequence 5′d(GCGT(10)GCG) was increased, the stability of duplexes formed with complementary DNA and RNA strands decreased and increased, respectively. This is due to the 3′-sulfur atom forcing the sugar to which it is attached and the sugar of the (n + 1) nucleotide to adopt a north conformation. This is the same conformation that ribose units exist in RNA, thereby enabling 3′-phosphorothiolate oligodeoxynucleotides to fit more easily into A-type duplex geometries. Alternating modifications were found to slightly increase the thermodynamic stability of oligodeoxynucleotide/RNA duplexes compared to consecutive ones due to more sugar moieties undergoing a conformational shift. The results do not show a saturation level in terms of the extra stability additional linkages bring; however, ΔT_m values do decrease as more alternating modifications are incorporated, thereby indicating that in a longer sequence saturation may be observed.

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